

Will India be the Sixth Country ?

FOR many years Indians have been deeply troubled by the question of whether or not to possess nuclear weapons. It will be recalled that it was India, through Prime Minister Nehru, who first proposed, in 1954, to the United Nations that there should be a standstill on weapons tests, and this set in motion the long chain of events that led to the Partial Test Ban Treaty. In 1957 Nehru declared: "We are not interested in making atom bombs, even if we have the capacity to do so". By 1964, Indian nuclear science had developed to the state that Prime Minister Shastri could say: "We have the capability of developing nuclear weapons, but we stand committed to use atomic energy only for peaceful purposes". And yet doubts remained that India could forever stay out of the nuclear club. There were clear signs that she was keeping certain options open when she refrained from signing the Non-Proliferation Treaty.

Within the past year there have been one or two exchanges in the Indian Parliament which suggest that something new is about to emerge. Interest in the use of peaceful nuclear explosions is sufficient for Mrs Gandhi to be able to say that India is now studying the feasibility of nuclear detonations for peaceful purposes. Copper-ore extraction by blasting could be the first experiment.

There is not a shadow of doubt that India is technologically capable of developing a nuclear bomb. There is a thriving programme for nuclear power generation including a power station being built in Madras which is of predominantly Indian design and construction. Fissile material presents no major obstacle as there is a good domestic supply of uranium and thorium. Plutonium production from the Madras power station will be subject to no safeguards. Thus, conditions in the near future will be ideal for the development of a nuclear explosive.

Can it be simply a peaceful device? Once the primary skills of designing a nuclear device have been mastered and the device tested in peaceful operations, it would be foolish to suppose that bombs cannot be made without further testing. Since both fission and fusion devices could be used in any extended programme of nuclear explosive engineering, there does not seem to be any reason why India could not develop quite an extensive armoury under the peaceful purposes umbrella. At present there are delivery problems but in the next few years an extensive space programme could easily provide a suitable vehicle.

The omens are not good. If India really wanted to use nuclear explosives purely for peaceful purposes, a well publicised approach to the Soviet Union (the most advanced nation in this technology) for assistance would have made more sense. The provision of explosives by the Soviet Union would have required a variance of the Non-Proliferation Treaty, but this might not have proved difficult to get.

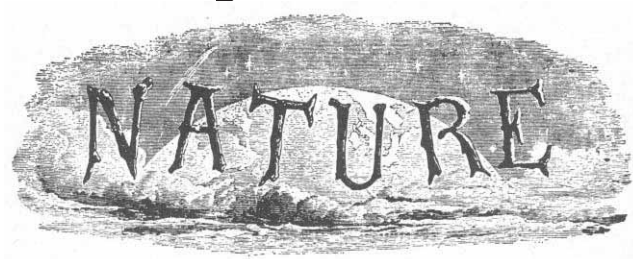
What inhibits a full scale military nuclear programme whether under 'peaceful' auspices or not? Cost is the central issue. Developed countries which have had a

nuclear weapons programme have found the economic impact a strain. There is little doubt that £1,000 million, at least, would be necessary for the most elemental force, and the Indian Gross National Product is only £20,000 million. One study, by the United States Arms Control and Disarmament Agency, labelled any attempt by India to go nuclear as "economically disastrous".

Even without the dreadful financial burden that nuclear weapons development would impose on India, there are many who believe, for strategic reasons, that India should not get herself embroiled in nuclear armament. There must be the strongest possible doubts that nuclear weapons could alter the military status of the country. Although the target is clearly China at present, there would be little value in a few nuclear weapons against a country so dispersed, and one can only imagine that nuclear weapons would be used in battleground situations on the borders and against a small number of military installations. It is difficult to see how India could win such an exchange and it would be better were she not to create a situation in which it could arise. Further, proliferation by India would be a positive step towards proliferation by several other countries.

One of the neatest and most thought-provoking of epigrams is due to Meister Eckhart: "The greatest power available to man is not to use it". It is distinctly appropriate to the nuclear age and highly relevant to India. This philosophy of "we can but we will not" has played a major role in preventing the spread of nuclear weapons to India in the past. One must hope that those who hold to a policy of self-restraint do not let their guard slip.

100 Years Ago

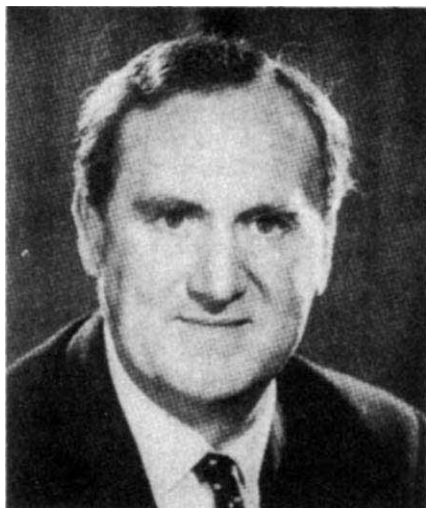


CANADA is doing its part toward the exploration of the Great West. Besides the surveying parties out on the route of the Pacific Railroad, it has special parties in the field in connection with the Geological Survey and the Boundary Commission. Mr. Selwyn, F.G.S., Director of the Survey, and Mr. R. Bell, F.G.S., are at work on the great regions watered by the North Saskatchewan, and Mr. Richardson on the other side of the Rocky Mountains in British Columbia. Mr. G. M. Dawson, Associate of the School of Mines, Geologist of the Boundary Commission, has just completed a survey of the Lake of the Woods and its neighbourhood, and is now exploring the plains westward of Pembina. All these parties are provided with the means of making collections in the botany and zoology of the regions explored.

From *Nature*, 8, 497, October 9, 1873.



Problems of Predicting Future World Trends



[Photo: Camera Press Ltd]

In this article, Sir Alan Cottrell, Chief Scientific Adviser to the British Government, examines some of the problems facing governments when they attempt to take into consideration, for policy purposes, predictions of the future such as have been made recently by several groups

If the trends in world population and economic activity continue into the distant future as they are at present, they may run into a limit some time in the next century, set by the Earth's presumed inability to provide the food, energy and materials that will be needed, or to cope with the volumes of pollution that may be created.

In the time since Professor Meadows's *Limits to Growth* was published, the MIT Group's assumptions, methods and conclusions have been discussed exhaustively by many contributors to the ensuing debate, and especially in the two outstanding works, *Study on Limits to Growth* by the World Bank, and *Thinking about the Future* by the Science Policy Research Unit of the University of Sussex. One must ask about the implications for the policies of governments; and from this point of view the projections of disaster derived from the model were surely made in the hope that they will be self-defeating prophesies: ignore them and they may come true; but act to ensure that they prove false.

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The model in its present preliminary state, however, could not possibly be a useful guide for policy-making. Several of the assumptions built into it are not demonstrably realistic and

could not be, in view of the gross inadequacy of world data for many of the factors that enter the analysis. We know too little, for example, about the potential food-producing capability of various lands, or of the economic and ecological problems of bringing virgin lands under cultivation. Again, the model does not give sufficient weight to the resourcefulness and technical ingenuity of mankind in overcoming material problems. Of course the invention and publication of world models, for the purpose of making self-defeating prophesies, may in itself be the latest and most sophisticated phase in the evolution of man's ability to adapt himself to and survive his conditions.

Not surprisingly, energy has come, since *Limits*, to occupy an increasingly more central place in the debate and, although it will be challenged there by food, if the growth in the world's population expected to occur in the next 30 years were to continue after then, it must be remembered that the achievement of higher food yields will itself depend largely on the increased application of energy, for example in the provision and distribution of fertilisers.

While governments must of course react to real, specific and immediate problems such as these, the generalised long-term projections of future world trends are too uncertain at present to form a basis for action by governments. But they are nevertheless too important to be left in an uncertain state, especially as the evolution of effective courses of action and, still more, of an endorsing

public opinion, could not occur overnight. Governments are beginning to recognise the need for a reliable and agreed foundation of natural knowledge in this field, which the science of the world's future has hardly begun to provide at present. This last point has been emphasised recently by Sir Kingsley Dunham in his Presidential Address to the British Association, when he said that "the identification of all the components of the life support systems upon which we depend, the investigation of their dynamic inter-relationships and the prediction of the effects of changes in one sector on the system as a whole are scientific objectives of far more than academic interest."

The factors of ignorance are enormous here. Large tracts of land are still uncultivated, particularly in Africa and South America, but it remains very unclear whether the botanical and geographic obstacles to their cultivation can be overcome. The best national yields per unit area of various crops can be some two to four times the world average, and experimental yields higher still, but much further study of the distribution of soil types and climatic conditions is needed before even scientific questions about the rigidity of these differences can be answered, quite apart from the economic aspects of the provision of fertilisers, insecticides and water, or of the distribution of food. Fish farming is in its infancy, but the separation of sunlight and nutrients, which keeps the oceans in a basic condition of low biological productivity, sets some technical problems the solution of which on a large scale seems extremely doubtful.

On pollution there is a lack of the data necessary to make firm and convincing projections

As regards mineral resources, although there is no ignorance of their measured reserves—some of which are not enough to last more than a few decades on present estimates—we have little knowledge of the extent of the low-grade or less accessible deposits upon which the world will be dependent in the future. On the question of pollution also, there are now some good mathematical models of the course and scale of various environmental effects, but there is generally a lack of the detailed and complex data necessary to make firm and convincing projections

from those equations. We are also quite ignorant of the effects, even on ourselves, of exposure to various amounts of pollution; of the thresholds, if any, below which there are no effects, or of aggravation at higher exposures. In particular there is a most difficult but important challenge to establish reliably the effects at low concentrations of the most widespread and persistent pollutants.

Moreover, all this is chiefly a scientific ignorance, an ignorance of the basic physical, chemical and biological facts about the capacity of our planet. It sets a huge scientific task, to provide in a

On the world scale, population growth undoubtedly deserves the highest priority for study

complete and irrefutable form the knowledge that will be needed as a basis for public action. The *Limits* study has been a benefit here, in exposing the need for more and better empirical data, as well as stimulating research into the systems analysis of future world trends. A lot of work is now being done on many of the issues that have been so raised by both private and publicly-supported groups in various countries, especially in Europe, America and Japan. The United Nations Organisation and its Agencies are making various studies and will hold its World Population Conference in 1974. The European Economic Commission has recently proposed to examine the trend of European development thirty years on, covering key sectors of society, to provide useful data for policy-making. An International Institute for Applied Systems Analysis has now been set up in Vienna, in which the United Kingdom participates through the Royal Society.

In fostering our own research efforts in the United Kingdom, it is important of course that the emphasis should take account of the wider activities and the contributions that we can make as well as reflect our own particular interests. On the world scale, population growth undoubtedly deserves the highest priority for study. There is a continuing need for more accurate projections of the growth and geographic distribution of world population, and for a clearer understanding of the social and economic forces which influence its rate of change. Again, there is no need to emphasise the importance of studies of world food needs and yields. The main focus here is the UN Food and Agriculture Organisation which is examining the longer term trends in its *Perspective Study of World Agriculture Development*, results from which should start to appear after 1974.

The United Kingdom of course shares with many other countries a great interest in local and world trends in energy supplies, and the government has accepted that in this, and other fields, it is important to extend to long time scales the analyses of availability and consumption of resources that are made by its departments in the course of their normal work, as well as to coordinate these interdepartmental studies so that the products of the analyses may be used effectively. Divisions of the Department of Trade and Industry concerned with energy and raw materials periodically investigate their future availability; and the data derived from long-term energy projections are used in general studies of long-term trends by the Systems Analysis Research Unit of the Department of the Environment. There are contacts here with similar groups examining energy problems in other countries and international organisations, as well as with the work of the OECD and EEC Energy Committees, of which the United Kingdom is a member.

In *Limits*, natural resources were lumped together into a single aggregate, but we need better estimates of future availability of individual major resources at various levels of cost; and to take account of the prospects for substitution and conservation through re-design, recycling and other technical changes. In this vast field of largely unknown factors it will obviously be sensible to identify and focus on those resources likely to be in shortest supply and for which economic substitutes are most doubtful. This is an area of direct interest to the United Kingdom. A study of the long-term availability of material resources, with special reference to the position of the United Kingdom, is thus being made by a joint unit of the Department of Trade and Industry and the Atomic Energy Authority. Again, the results will be used in the modelling studies of the DOE Systems Analysis Research Unit.

Resources necessary to keep pollution permanently at a safe level are well within the means of industrialised countries

On pollution, the position taken in *Limits* has been severely criticised in the literature and there is little doubt that the diversion of resources necessary to keep pollution permanently at a safe level—given the scientific knowledge necessary to define this competently—is well within the means of industrialised countries. But it remains a field of great scientific challenge, and the DOE together with other departments and

the research councils is sponsoring a large research programme, which includes scientific investigations into the effects of various pollutants on health and the environment, as well as economic analyses of the costs of controlling pollution. Particular attention is being given to quantitative studies of the pathways and effects of pollution, as well as to the costs of reaching and holding agreed standards of environmental quality in the presence of rising output and consumption. The importance of more accurate and complete monitoring of pollution levels and of the effectiveness of controls is now fully recognised. The results of these efforts, while intended to be applied directly to policies for the control of pollution, should also provide better data for models of the ecological aspects of world trends.

Technological change is, by its very nature, one of the most chancy factors to project into the future, but it would be as rash to ignore its contributions as to bank on their power to rescue the world from all future material difficulties. A lot can be done, however, in analysing costs and time scales of substitutions for various resources. Problems of this kind are being studied in government departments, as well as in industry and the universities.

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To pull these various studies together and to determine how the different effects interact with one another, overall models of the future trends have to be made, as was demonstrated by the MIT Group. Dynamic modelling is a way to take account of the many, diverse and inter-related factors that determine future trends and so do justice to the complexity of the problems. The DOE Systems Analysis Research Unit is working on such models, using data from the other studies and outside sources. But it may be beyond the scope of any one group, or even country, to provide results quickly, comprehensively and reliably, especially as the early work in this field has pointed up the paramount importance of a thoroughly scientific and objective approach to the matters under consideration. Fortunately, however, it is a field in which the work of different groups can contribute constructively to an overall total progress. By the efforts just described, the United Kingdom should reach a position where it is not merely willing, but also equipped, to play its part in wider international studies as and when these emerge.

OLD WORLD

Dual Support System Questioned

THE dual system of supporting research in Britain comes in for close analysis in the Social Science Research Council annual report published last week (*SSRC Annual Report*, HMSO, £0.47).

Professor R. C. O. Matthews, the council's chairman, argues that the dual system, where research funding comes from both research councils and the University Grants Committee, is, in its present form, limited in its usefulness. In particular, the system has its drawbacks when a particular area of research needs a stimulus. The usual procedure is to make it known that applications in the appropriate field will be looked on with favour. But such a system, says Professor Matthews, is contrary to the dual support system in that university research grants from a research council should not in normal circumstances pay for the salary of the principal investigator or pay for buildings. The councils can pay for senior research officers but the "absence of tenure makes such appointments unattractive to those who are able to secure university teaching posts".

The nub of Professor Matthews's argument is that shortage of finance for ancillary facilities or research staff at the relatively junior level may not be the chief obstacle to expansion of research in a particular area. "Where this is so, announcement that research grant applications in the area will be welcomed may not be very effective in promoting an expansion of research in it."

The report also raises the problems which will be caused by the differing rates of expansion of the science vote (not more than 3% a year in the next five years) and the rate of expansion of the universities' recurrent expenditure (5.25% a year). The dual support system, argues Professor Matthews, will not be put under any undue strain in the next quinquennium, because of the tightness of university budgets. The research councils will have to continue to support, for as long as they last, research programmes which previously a university would have been expected to take over after an initial period.

Finance from the UGC, says Professor Matthews, is geared to teaching needs and "research emerges to a large extent as a byproduct of teaching". And this is likely to become more pronounced in the years ahead.

Another side effect of the dual system of support is that expansion in particular disciplines will be tied to an increase of

students in that discipline because UGC finance is directly related to student numbers. Thus student preferences, argues Professor Matthews, will have an influence in the long term on the areas in which research is carried out.

Professor Matthews also argues for the rate of increase of the Social Science Research Council budget to be kept well above the 3% overall increase in the science vote. Rates of growth of different subjects can only be expected to be similar if there was equilibrium initially, but as the SSRC at present only receives 2% of the total vote a strong case can be made for increasing it to at least 4%.

The expansion of the science vote and the UGC budget in different areas could well lead to a gap opening up between the numbers of postgraduate places available at universities and the studentships available to fill these places.

This real fear has, in fact, been recog-

nised by the Advisory Board of the Research Councils which has set up a joint committee with the UGC to keep these matters under close attention. But as far as the SSRC is concerned there is, at present, no worry about a divergence of views between it and the UGC. Professor Matthews says that the views of the SSRC and the UGC are closely in line and that the SSRC intends to increase the numbers of studentships available "at no less than the percentage rate at which the number of places in universities is increasing". The only factor which will jeopardise this pledge is an insufficient rate of growth of its budget.

In 1972-73 the SSRC allocated £2.45 million in research grants to be compared with £1.66 million the year before. The council also awarded 2,343 postgraduate awards to a total value of £2.38 million, an increase of 180 students and £270,000 over 1971-72.

MARINE POLLUTION

Dearer Petrol, Cleaner Seas

COMPLETE elimination of intentional pollution of the sea by oil, chemicals, garbage and sewage is the aim of a four-week conference just started in London.

One new convention is up for discussion, while an amendment to an existing 1969 agreement will allow countries to take action when their coastlines are threatened by tanker accidents that occur outside their territorial waters.

Proposals for the control of oil and chemical pollution include designing future tankers with separate ballast tanks large enough to obviate the need to fill cargo tanks with seawater as ballast for the return trip. One way is to build ships with double bottoms.

For existing tankers it is planned to transfer to the new convention the agreements made in 1969 for limiting the amount of oily water that can be discharged by a tanker. Other proposals include installing meters on tankers to record precisely how much oil is loaded and unloaded, and how much discharged on the voyage, while masters and owners may in future be required to keep oil record books.

A further radical proposal is that countries where tankers dock should be able to prosecute the owners and master for pollution caused on any part of the voyage. At present, tankers can only be prosecuted for pollution caused in

territorial waters.

These tough measures, if agreed, could go a long way towards improving the state of the oceans, as, for the first time, the convention covers chemicals.

But, as usual in pollution matters, there is a disagreement between the developing countries and the richer nations as to how forceful the new convention should be. All these proposals cost money. Building tankers with segregated ballast tanks could add between 4% and 8% to the price.

Further complicating factors are the need for several countries to ratify these conventions before they come into operation. The 1969 amendments to the 1954 Convention for the Prevention of Pollution of the Sea by Oil have still not been ratified by enough countries for them to come into effect.

In addition, the United Nations Law of the Sea Conference planned for Santiago or Vienna next year may preclude any real decision being taken in London this month. It has already been agreed that decisions taken at this meeting will not prejudice next year's conference. And as a number of developing countries, notably the Latin Americans, will be pushing for 200 mile patrimonial seas and for the right to set their own pollution standards in these waters, it is possible that hard decisions could be deferred.

Short Notes

Education

ONLY 3.7% of Britain's expenditure on educational research goes on further education. This is one of the facts revealed today in a report, *Resources for Educational Research and Development*, published by the National Foundation for Educational Research.

The percentage of the total education budget spent on research and development on education in Britain (0.12%) is higher than in some European countries. In the United States, however, \$250 million was spent in 1968, amounting to 0.31% of the total available for education.

The league table of sponsors of research into education is, not surprisingly, headed by the Schools Council which during 1968–70 spent £609,000 a year on this type of research. The Department of Education and Science comes next with an annual expenditure of £464,000. The Nuffield Foundation, the Social Science Research Council, the Leverhulme Trust, the National Foundation for Educational Research and the Ford Foundation complete the table of supporters. The report points out that there is uniformity in the machinery within these organisations for dealing with applications for grants but that the outcomes of research applications may vary widely. The report adds that the reason why applications fail to win approval is not always clear and "it is impossible to establish how decisions are made concerning research applications".

UKAEA

NUCLEAR fusion and plasma physics on a European scale were boosted this week with formal signing of a contract of collaboration between the European Atomic Energy Community (Euratom) and the United Kingdom Atomic Energy Authority.

The contract merges the authority's fusion work into the existing five-year programme of Euratom which is due to run until 1975.

The aim of the programme is to build large fusion experiments and finally a prototype fusion reactor. Studies of a large Tokamak experiment are currently under way at Culham, the UKAEA's fusion laboratory, led by Dr Paul Rebut of the French Commission de l'Energie Atomique. A detailed proposal for construction of the large experiment is likely to be put to the Council of Ministers in about a year's time. The cost will probably be between £10 and £20 million.

This week's contract also provides for Euratom to pay one quarter of the cost of the existing British research programme—currently running at £17 million over three years.

The signing of the Euratom contract

was announced by Sir John Hill, chairman of the UKAEA, introducing the authority's annual report. Commenting on the recent incident at Windscale when 34 men were contaminated with radiation, Sir John said that the accident was "a very small one". It was, he said, the sort of incident that would cause no comment if it occurred in an ordinary chemical factory.

Mercury Pollution

THE Organisation for Economic Co-operation and Development has recommended that all OECD countries should make a concerted effort to reduce the discharge of mercury compounds into the environment. The organisation has also called for governments to exchange information on mercury usage and on measures taken to abate pollution. The next step according to the OECD is to attempt to obtain agreement on the maximum mercury levels to be allowed in industrial discharges or products.

Ten thousand tonnes of mercury were produced world wide in 1969 and the OECD countries produced 66% of this. By 1972 this output is estimated to have increased by 20%. But only about 20% of the mercury is recycled.

Mercury is used mostly in electrolysis in order to produce chlorine and caustic soda. In agriculture it is used as a bactericide and fungicide for seed. As far as this latter use is concerned, the discharge of mercury into the environment can be halted if some of the newer (mercury-free) seed dressings are brought into common use.

Research Spending by MAFF

THE programmes of all agriculture, fisheries and food research establishments are being reviewed to highlight those projects which are of specific interest to the Ministry of Agriculture, Fisheries and Food. The ministry's first annual report on its research and development activities, published this week, reveals that public expenditure in this field was £40 million in 1972–73. Seven and a half million of that was money transferred from the Agricultural and Natural Environment Research Councils' budgets to the ministry. But this money has been spent entirely with the research councils from which it came, and the report reveals that the whole of the transferred funds will be spent on commissioning work from the research councils up to 1976.

The report also calms some fears expressed last year when the white paper *A Framework for Government Research and Development* was published. "Commissions will not be confined to short-term applied work," the report states. "Depending on the objective, they may embrace basic as well as applied research and be of any duration".

Details of the ministry's revised

organisation for fisheries research are given, with the information that the emphasis on fisheries work has changed during the year. Entry to the EEC has resulted in more work on inshore resources to ensure that the interests of British fishermen are fully protected; next year's law of the sea conference may radically change fishing limits and work is underway to predict the effects on the British industry of the changes under consideration; and work on fish disease, monitoring marine pollution and reviving the North Sea herring fisheries have all received a boost.

Facts and Figures

STATISTICS released last week by the Department of Education and Science reveal that the numbers of undergraduates and postgraduates in British universities have increased from 114,758 in 1957–58 to 258,640 in 1971–72. In the same period the number of professors has increased from 1,505 to 3,487 with lecturers increasing in numbers from 7,336 to 19,192. But senior lecturers have enlarged their ranks threefold in the fourteen year period. There were 6,032 of them in 1971–72, but only 1,925 in 1957–58.

Guinea Pigs

ANYONE interested in a ten-day holiday—with pay, or at least pocket-money—should contact the Common Cold Unit in Salisbury. Not only is the unit prepared to feed and bed volunteers during their stay but travelling expenses will also be met, and a princely sum of £0.35p a day will be doled out to each volunteer. And, believe it or not, the chances of catching a cold are only one in three. The infections, if they do occur, according to the holiday brochure put out by the Medical Research Council "are normally minor and brief".

It all sounds too good to be true. The vacancies at the holiday home in Salisbury are in October. If any reader feels that the onset of the new term with hordes of keen graduate students is too much then the Common Cold Unit will be glad to provide a haven. There is no reason to be lonely, for volunteers are put up in "comfortable well-heated flats—in pairs or threes".

Nature in 1974

As from January 1974, *Nature* will be published once a week, as announced on August 17 (*Nature*, **244**, 381). The subscription will be £22 in Britain and elsewhere except United States and Canada where it will be £28. New orders placed before the end of the year, however, will be accepted at this year's prices of £16 and £20 respectively.

NEW WORLD

Brighter Outlook for Technology Assessment

by our Washington Correspondent

THE fortunes of the hapless Office of Technology Assessment (OTA), which has been afflicted for more than a year by Congressional footdragging and bizarre strokes of bad luck, brightened a little last week. It is now likely that the office could even be in business by the end of the year. But even so, it will end up with only about half the money that its backers originally wanted, it will not be able to get down to much serious work before next summer, and it still has a few dangerous waters to negotiate.

The one bright spot is that a compromise is understood to have been reached last week on a matter which, although entirely unrelated to the OTA, has been holding up funds for the office and even threatening to torpedo it entirely. The matter in question is the west front of the Capitol building.

It was, in fact, exactly a year ago that Congress decided to give itself some badly needed technological expertise by passing a bill to establish the OTA. Designed to provide analysis and advice to Congress on issues and legislation involving science and technology, the office still has no funds, and therefore no director and no staff—in other words, it still does not exist. The problem is that although the OTA bill was passed a year ago, a separate vote is required to provide money for the office, and so far attempts to tack OTA money on to appropriations bills have been spectacularly unsuccessful.

The first such move came last summer, when Senator Edward M. Kennedy managed to get the Senate to agree to put \$289,000 into the supplemental appropriations bill—a bill providing money to a variety of agencies for the 1973 financial year (which ended on June 30). But there was no money in the House version of the bill and the conference committee got bogged down over another matter, namely a provision in the bill forcing President Nixon to stop bombing Cambodia. Eventually, the bill was passed with only a couple of weeks of the financial year to go, and the OTA funding had been dropped because, it was argued, there was not enough time to spend it wisely.

Undaunted, OTA's supporters turned to the appropriations bill which provides funds for Congressional activities in the 1974 financial year, and they again ran into trouble. The Senate version of the bill contains some \$3.9 million for OTA, but the House version contains

nothing, and the matter has been embroiled in another longstanding dispute, this time over the crumbling west wall of the Capitol. In short, the House bill contains money to extend the wall to make room for more offices, while the Senate version contains money simply to restore the wall. It was feared that the OTA money would get lost in trade-offs in the conference committee, but the Congressional leadership has apparently now come to an agreement on the west front and it is hoped that a final version of the bill can be passed and signed into law by early November.

In any case, the OTA funds will be reduced by at least a third, since the financial year will by then be four months old. And that would give the office only half of the \$5 million originally envisaged for its first year of operation. It will, however, allow a director and some staff to be appointed. Emilio Q. Daddario, the former chairman of the House subcommittee on Science, Research and Development, is still a safe bet as director.

Once OTA is off and running, one of the first items on the agenda will be the appointment of an advisory committee to provide policy guidance, and panels

of outside experts to study some specific problems. Energy research and development, food technology, ocean resources and solid waste disposal are among the items being considered for the panels to study.

The office already has a 12-member board, consisting of six Senators and six Congressmen, to establish policies and provide control over its activities. Kennedy has been elected chairman of the board, and John Davis, a congressman from Georgia who succeeded Daddario as chairman of the subcommittee on Science, Research and Development, is the vice-chairman. It has, in fact, been rumoured that part of the OTA's financial troubles have been due to Kennedy's connection with the enterprise—essentially, the reasoning is that some Congressmen are reluctant to provide him with another power base from which to launch a possible Presidential bid. But Congressional staff members concerned with the OTA, in both the House and the Senate, have rejected such an assertion. Whatever the truth of the matter, the delay has certainly diminished Kennedy's influence because his term of office expires at the end of next year.

NIH

Victory or Vetoes

by our Washington Correspondent

IT is now almost certain that President Nixon will veto the appropriations bill which contains money for the National Institutes of Health, when it eventually emerges from the Congressional mill. In June, the House of Representatives added some \$1,300 million to Nixon's budget request for the departments of Labor and Health, Education and Welfare (HEW), and last week the Senate approved an even larger amount—it added \$1,800 million to the president's budget. Thus, when a conference committee has sorted out the differences in the two versions of the bill, Congress is sure to end up voting at least \$1,500 million more than Nixon wants to spend.

Although the bills were passed by large margins—347 votes to 58 in the House and 79 votes to 9 in the Senate—it is questionable whether supporters of the bills could muster the two-thirds majority needed to override a presidential veto in both the House and the Senate. Last year, for example, Nixon twice vetoed HEW appropriations bills

that had been passed overwhelmingly, but several Republicans bowed to White House pressure, voted to sustain the vetoes, and tipped the balance.

In any case, the bill passed last week by the Senate would add huge helpings to the plate of the National Institutes of Health—it contains \$1,882 million for the research institutes of NIH, which is some \$350 million more than the Administration wants to spend, and \$140 million more than the House voted.

Even if the bill is not vetoed, or if the veto is overridden by Congress, the Administration could simply do what it has done in the past few years—refuse to spend the extra money. A court suit filed last month by sixteen organisations, headed by the National Association for Mental Health, two states and fifteen individuals, could, however, tie its hands in that respect. The suit charges that the Administration acted illegally last year in refusing to spend some \$126 million voted by Congress for mental health and alcoholism programmes. If the Administration loses the case, it would be forced to release the money immediately, and it would probably not be able to impound funds in the future.

NEWS AND VIEWS

Swine Vesicular Disease and Coxsackie Infection

THE appearance of swine vesicular disease in England in December 1972 caused much concern because of its similarity to foot-and-mouth disease in pigs. A differential diagnosis was achieved in a few days, largely owing to experience gained at the Animal Virus Research Institute, Pirbright, during a short-lived outbreak in Italy in 1966 and a later series of outbreaks in Hong Kong during 1971–72. In the earlier outbreaks, the virus was identified as an enterovirus which differed from other members of the group already found in pigs both serologically and in its ability to infect baby mice. Its relationship to enteroviruses of other species, including man, was not investigated at that time although it is realised that its properties were like those of the Coxsackie group.

Two communications in this issue of *Nature* (see pages 314 and 315) continue the story. Graves at the Plum Island laboratory has carried out exhaustive cross-neutralisation tests against Coxsackie antisera and has found a strong link between the swine virus and Coxsackie strain B5. At Pirbright, Brown *et al.* have found by immunodiffusion tests that the two viruses, although sharing a common antigen, possess also an antigen specific to each.

These findings point to an interesting relationship between Coxsackie infection in man and swine vesicular disease. Neither in the laboratory at Plum Island nor at Pirbright has overt disease been produced in pigs inoculated with Coxsackie B5 virus. At Pirbright and

in at least one other laboratory, however, several research workers handling swine vesicular disease virus became ill with clinical signs like those of Coxsackie B5 infection. Using the tests described by Brown *et al.*, these infections have been confirmed as being caused by swine vesicular disease virus. It is interesting, however, that no cases were reported in the field among staff dealing with the outbreak of the disease in Britain.

This interrelationship between the two viruses prompts speculation that the origin of swine vesicular disease might lie in some such sequence of events as has been put forward to account for the appearance of new human strains of influenza which may have been derived from infection in swine. In the case of swine vesicular disease it seems possible that inapparent Coxsackie B5 infection may have taken place in swine and that suitable conditions have arisen for the development of a strain of virus which produces overt disease in swine but has a lower infectivity for man.

This relationship between Coxsackie B5 and swine vesicular disease seems to be yet another illustration of the interspecies transfer of a virus. Such findings should stimulate interest in the relationship between viruses within a single taxonomic group but which affect different species with a variety of clinical manifestations. A study of these interrelationships may lead to a better understanding of the whole question of virus/host interaction.

From a Correspondent

Intraplate Earthquakes—Thrusting Everywhere

THERE cannot be a reader of *Nature* who doesn't know by now that earthquakes mark boundaries between the rigid plates which cover the Earth's surface. Yet there are still a few earthquakes which do not lie on or near these boundaries; for instance, in Eastern North America there have been large quakes in historical times, Australia and India have recently suffered extensive damaging quakes and even Britain every few years experiences a modest jolt. Instrumental recordings point to several quakes a year in the oceans away from plate boundaries. What can be learnt from these events?

Sykes and Sbar of Lamont-Doherty Geological Observatory, New York, report in this issue of *Nature* (see page 298) on a comprehensive study they have recently made of about eighty intraplate earthquakes globally distributed. Using techniques for determining focal mechanism which were highly successful in the late nineteen sixties in helping to establish plate tectonics, the authors have been able to determine the pattern of displacement of the rocks in the vicinity of the seismic source at the time of the earthquake. These patterns are quadrantal distributions of movement towards and away from the observer depending on the orientation of the fault plane and slip vector.

A global plot of these would yield pure confusion for the following reason. If a rod is subjected to extreme tension

or compression fractures would appear by earthquake-type processes, but the orientation of the fault-plane would be controlled very strongly by preferred planes of yielding. The only thing that can be said with some certainty is that the normal to the fault surface will be roughly at 45° to the axis of maximum tension. Thus from a wide variety of focal mechanisms that the rod would display one should expect to see a consistency in the tension or compression axis—an axis which is easily deduced from the focal mechanism. Sykes and Sbar have deduced from their analysis that a large majority of the quakes that they consider have a horizontal compression axis. This is a very important result indeed and confirms work on separate earthquakes in the past year or two and also measurements of *in situ* stress that have been made by stress gauges in a few places. Furthermore the axes of the ambient compression for adjacent observations seem to be quite consistent, implying (in the absence of aliasing) that there are broad scale patterns of compressive stress over the Earth's surface.

It is tempting to try and go on from these observations to understand more about the forces driving plates. This is a problem for which no clear solution is yet in sight. Plate tectonics was conceived as a kinematic model of motion and a dynamical explanation which produces

broad agreement in the geophysical community has yet to be proposed. The gravitational effect of sinking slabs, pressure effects from mid-ocean ridges, deeper convective patterns as manifested either by broad scale motions or by narrow plumes are all under study, but the nut is a tough one. Sykes and Sbar look at these processes and compare their results with the implications of separate processes. In no case is there anything like good agreement, so they refrain from coming down in favour of any one explanation. Instead, they have provided geophysicists with a new and powerful constraint on dynamical models of plate tectonics, and in the long run this is probably more important.

D. D.

Antigens on Trophoblasts

MODERN immunology has more than its share of riddles and paradoxes, and one of the most intriguing of these is the failure of the mother immunologically to reject her foetus, in spite of the fact that they have—in outbred animals and humans—a relationship that is biologically akin to that of an allograft and its host. The solution to this puzzle is important for reasons which go beyond satisfying simple immunological curiosity: for one thing, an understanding of the methods nature uses to prevent foetal-allograft rejection could go a long way in helping to solve the rejection problem which plagues the transplant surgeon.

Inevitably, an array of explanations has been put forward; these include depressed maternal immune responsiveness, immunological 'enhancement' mediated by serum blocking factors, placental barrier function and weak foetal immunogenicity. None of these theories, however, commands wide general acceptance.

The presence of antigens on trophoblast cells is a case in point. It is known that the mother can, and often does, make antibodies to some of the alloantigens of her foetus even though maternal and foetal circulations are entirely separate. To resolve this problem much attention has been paid to the trophoblast layer of the placenta as it is the only location where there is mutual surface contact between maternal and foetal tissues. Hence the special interest in the subject of antigens such as histocompatibility or blood group antigens on trophoblast cells. In short, is the trophoblast immunologically neutral or can it sensitise the mother to histocompatibility and blood group antigens? Most studies have been negative in detecting histocompatibility antigens on trophoblast cells, but enough have been successful to keep the issue continually embroiled in controversy. One popular notion is that antigens on trophoblast cells are 'masked' by a thick sialo-mucinous glycocalyx. The experiments in which this cell coat has been enzymatically removed with neuraminidase have not, however, yielded consistent evidence of the exposure of antigens.

On page 329 of this issue of *Nature*, Loke and Ballard address themselves to the problem of blood group antigens (type A) on human trophoblast cells. The authors use three different techniques to try and detect such antigens: first, a mixed agglutination method using trophoblast cells coated with anti-A serum and group A red blood cells; second, an antiglobulin method using group A red cells and trophoblast both being coated with anti-A with a rabbit anti-human globulin acting as a cross-linking

bridge molecule; and, third, an electron microscopic method using a coating of ferritin-labelled anti-human globulin antiserum on cells previously incubated with anti-A antiserum. The second method yielded an unequivocally positive result and the third, though permitting some visualisation of 'A' antigens, suggested they were present in small amount or density. Loke and Ballard speculate that, because the surface density of antigens on trophoblast cells is low and their spacial topography such, only very sensitive methods will be successful in their detection. Low antigen density (or antigen inaccessibility) may result in insufficient antibody being taken up to form stable bridges directly with the indicator cells in the mixed agglutination method. Addition of another link introduced in the form of an antiglobulin, however, results in visible agglutination between trophoblast cells and indicator red cells.

The question now is what, if anything, is the biological importance of low antigen density on trophoblast cells? Loke and Ballard suggest that since antigenic density of target cells may be an important factor in the phenomenon of immunological enhancement, the fixation of antibody on surface antigens which are sparsely distributed may lead to a 'blocking' or enhancing effect, thus helping to ensure survival of the foetus. As with so many biological problems for which there are various and conflicting solutions it seems that none of them will prove uniquely to be correct.

From a Correspondent

Cosmological Coincidences

Two cosmological coincidences are discussed by Cavallo on page 313 of this issue of *Nature*. One of them is the Dirac relationship between fundamental constants.

It is well known that in situations where well-founded theoretical arguments are difficult, a dimensional argument (if it leads to a unique answer at all) will give an answer which is not usually out by a large factor. The reason is that nature's dimensionless numbers do not differ by many orders of magnitude from unity. Even the sign structure constant (about $1/137$) is something of an exception.

Two very striking exceptions are numbers of order 10^{40} . One is the ratio of electromagnetic repulsion to gravitational attraction between two electrons. The other is a dimensionless combination involving Hubble's constant (which determines the rate of the Universe's expansion and is thought to be related to its age so that the combination could be thought of as a way of expressing the age of the Universe in "natural" units).

The second of these numbers is not well enough determined to refute the hypothesis that it is identically equal to the first. This is a striking coincidence. It was proposed by Dirac in 1937 that strict equality might hold between the two numbers and that gravity weakens to maintain the equality as the Universe gets older.

The second coincidence was expressed dramatically in 1969 in a paper by Hawking (Cavallo's reference 3) called "Is the Universe Rotating?". It is not a meaningless question in spite of the rejoinder "Relative to what?" because rotation produces local mechanical effects. It is a coincidence that the frame of reference in which there are no such mechanical effects is the same as that in which the distant galaxies are not rotating around us.

The effect of Hawking's work was to exhibit the close limits in which this could be said to be experimentally known. This coincidence is often asserted as a principle (Mach's principle) and has motivated much cosmological research — including general relativity.

Cavallo proposes that to explain the first coincidence one should abandon belief in the second. He exhibits a class of rotating cosmological models and shows how Dirac's relationship emerges in a natural way if the Universe is endowed with the right non-zero angular momentum.

The interest of this lies in a third coincidence, that the angular momentum which the Universe needs for the theory satisfies an empirical relationship elucidated by Cavallo between angular momentum and volume which seems to be satisfied by fundamental particles, stars and stable galaxies.

The concept that our Universe is rotating is anathema to many astronomers, and no doubt further work on establishing lower bounds on the rotation of the Universe will be stimulated by a desire to refute Cavallo's suggestion.

P. E. R.

NEUROBIOLOGY

Twenty Years of Neurosecretion

from a Correspondent

In opening the Sixth International Symposium on Neurosecretion at the Royal Society (September 17–21) Sir Francis Knowles (King's College, London) reviewed the progress which had been reported at previous meetings and developed themes which were to be discussed in the ensuing eighty papers.

Perhaps one of the dominating themes of the meeting was the question of whether exocytosis is the release mechanism for neurosecretory neurones. W. W. Douglas (Yale University Medical School) reviewed the evidence for exocytosis in the neurohypophysis and pointed out that the difficulty of capturing the event in electron micrographs may be a function of the rapidity and rarity of the process. This argument was taken up again during the meeting; for example, D. W. Lincoln (University of Bristol) suggested that the milk-ejection reflex in the rat is associated with the release of only one in a thousand neurosecretory granules. Nevertheless some beautiful micrographs of freeze-etched/freeze-fractured neural lobes in which apparent exocytotic pits could be seen were shown by J. J. Dreifuss and colleagues (University of Geneva) and W. B. Watkins and colleagues (University of Auckland). The occurrence of the extragranular pool of hormone in the neurohypophysis was questioned; it seems that the 'readily-releasable' pool may consist of granules which are close to the membrane of the nerve endings and thus readily available for exocytosis.

Quite understandably a great deal of attention was focused on the synthesis and transport of the neurosecretory granule in what L. Vollrath (King's College, London) referred to as 'classical' neurosecretory neurones: the mammalian hypothalamo-neurohypophyseal system. D. Picard and collaborators

(Faculty of Medicine, Marseilles) presented electron-microscopical evidence for the formation of granules on the opposite side of the Golgi from that producing lysosomes. They also drew attention to evidence for a glycoprotein present in the periphery of the neurosecretory granule. This demonstration prompted N. A. Thorn (University of Copenhagen) to mention that his group had found hexosamine in a granular membrane fraction, and B. T. Pickering (University of Bristol) to say that glycoprotein was seen after polyacrylamide electrophoresis of neural lobes and that this too seemed to be preferentially located in the granular membrane. This protein looks as if it might feature more prominently in the next symposium.

It is now clear that neurosecretory material is transported along the axons quite rapidly (about 50 mm d⁻¹). The problem of the apparent slow transport of the minor neurophysin component may have been overcome by the evidence presented by B. T. Pickering and colleagues that it may be a metabolic product of one of the principal proteins and arise *in granulo*. This also means that the rat now has two neurohypophyseal hormones and two neurophysins. Evidence for the mechanism by which neurosecretory granules are transported is still sparse. J. Flament-Durand and P. Dustin (Free University, Brussels) presented some excellent electron micrographs and autoradiographs showing that administration of colchicine inhibits transport in the hypothalamo-neurohypophyseal system of the rat. There were, however, no apparent changes in the appearance of the neurotubules in the treated animals. Preliminary evidence from F. Grainger and J. C. Sloper (Charing Cross Hospital Medical School, London) indicates that the neurones of the hypothalamo-neurohypophyseal

system in saline-stressed rats and in animals with diabetes insipidus may be richer in neurotubules than in normal rats. It seemed to be agreed that, although there is no concrete evidence associating granule transport and neurotubules, no alternative hypothesis seems to be available at present.

In the past few years evidence for the existence and location of hypothalamic releasing substances has been largely biochemical and pharmacological and was reviewed by L. Martini (University of Milan). Vollrath pointed out in his concluding remarks, however, that the papers at this symposium showed that the "morphologists are rapidly catching up". J. Barry and M. P. Dubois (Faculty of Medicine, Lille) described the presence of cell bodies, scattered mainly in the preoptic area of guinea pigs, which react with antibodies to synthesise luteinising hormone-releasing factor and can be visualised with an immunofluorescent technique. Axons from these cells can be seen projecting towards the median eminence where there is an abundance of fluorescent material. Similarly, H. Vullings (University of Utrecht) used autoradiographic methods to show that material incorporated in the preoptic nucleus of frogs is transported to the outer zone of median eminence and that the activity of this system is affected by light.

There was great interest in the report by C. A. Mason and R. S. Nishioka (University of California, Berkeley) of the application to neurosecretory systems of a technique for demonstrating the course of neurones by iontophoresis of cobalt chloride into the cut ends of axons. These workers have identified the course of the neurosecretory fibres in locusts and there was great hope expressed by participants that the technique may help in the location of the cell bodies whose axons terminate in the mammalian median eminence and which probably synthesise releasing factors.

The establishment of the neurosecretory neurone as an electrical entity was reviewed by B. A. Cross (University of Bristol) who has found that its bioelectric properties are the same as those of other neurones. This property can be utilised for the identification of the course of neurosecretory neurones by the generation and recording of antidromic action potentials. R. G. Dyer (University of Bristol) described how this technique can be used to demonstrate that neurones in the preoptic area of rats project to the median eminence and the audience was tempted to associate these cells with those demonstrated by Barry and Dubois. The possible relation between the electrical and endocrine properties of neurosecretory neurones was illustrated by

D. W. Lincoln (University of Bristol) who described the correlation between activity in supraoptic and paraventricular cells and the release of oxytocin in suckling rats. Just before such a release there is a tremendous burst of activity in the hypothalamic neurones.

In his opening address Sir Francis Knowles mentioned the "enigmatic ependymal elements in the ventrolateral walls and floor of the hypothalamus". Several contributors focused on these liquor-contacting cells which may be monitoring changes in the cerebrospinal fluid (CSF). For example, D. E. Scott (University of Rochester School of Medicine, NY) showed that [^3H]TRF is rapidly taken up from CSF possibly by such ependymal cells. O. C. McKenna and J. Rosenbluth (New York University) reported that there are two types of aminergic liquor-contacting cells in the toad, one in the preoptic area and the other along the infundibular recess. Interesting similarities and differences between fishes and reptiles were highlighted by I. Vigh-Teichmann (Semelweis University of Medicine, Budapest). She has found that preoptic neurosecretory neurones projecting towards the median eminence in fishes also have liquor-contacting dendritic terminals whereas in reptiles this pathway seems to be in two stages with possibly synaptic contact between CSF-contacting neurones and those which project towards the median eminence.

During the meeting a pattern of integration of the neurosecretory systems began to emerge, with a growing awareness of the functional association of the aminergic and peptidergic elements of the hypothalamus. There seems to be a wide range of aminergic neurones connecting the hypothalamus with "higher centres". In addition to the recognised brain neurotransmitters such as noradrenaline, serotonin and dopamine, K. Fuxe and T. Hökfelt (Karolinska Institute, Stockholm) reported the presence of terminals containing adrenaline. K. Fuxe also reviewed evidence that dopaminergic neurones in the median eminence may play an inhibiting part in the release of luteinising hormone releasing factor from peptidergic terminals and noradrenergic neurones might facilitate such release. This sort of interaction was mentioned frequently during the meeting. The occurrence of ascending peptidergic fibres was also recognised. G. Sterba (Karl Marx University, Leipzig) demonstrated neurones with cell bodies in the supraoptic nucleus which sent fibres to extrahypothalamic structures (for example, lamina terminalis and septum) and suggested that peptidic neurohormones might be involved in the generation of emotions by effects in the limbic system.

Sir Francis Knowles mentioned that at the first symposium in Naples in 1953, 60% of the papers dealt with invertebrates. In the present meeting the proportions were more than reversed, although everybody was aware of the great debt that vertebrate neurosecretion owes to the observations of investigators working with invertebrates. In reviewing recent advances in invertebrate neurosecretion B. Scharrer (Albert Einstein College of Medicine, New York) pointed out evidence for synapse-like interactions between neurosecretory neurones and other cells, where the mediator is likely to be a peptide. Thus another useful criterion for the definition of a neurosecretory neurone—that it ends in a neurohaemal organ—loses its generality. It was obvious that the meeting could not agree on a watertight definition of a neurosecretory neurone which has slightly different connotations to different people.

ENTOMOLOGY

Social Insects

from a Correspondent

THE Seventh Congress of the International Union for the Study of Social Insects (September 10–15) was held for the first time in Britain, and attracted nearly twice as many members as the previous congress in Berne (1969). Studies on pheromones and on caste determination in social insects provided the dominant themes of the meeting.

The wealth of chemical studies on ants was illustrated by M. S. Blum (University of Georgia) who noted that at least forty-seven hydrocarbons have so far been identified in the Dufour's gland of ants. Many of the exocrine secretions of ants contain compounds unrecorded elsewhere in the animal kingdom. The Dufour gland secretion is

commonly used as part of a defence alarm-recognition system and Cammaerts-Tricot (Free University, Brussels) reported that the ant, *Myrmica rubra*, uses the secretion of this gland to attract nest mates to a source of disturbance. A mandibular gland secretion which incites the ants to sting any intruder may also be produced, and if the danger persists, additional nest mates are recruited with the aid of a trail pheromone produced by the poison gland. The mechanisms governing the perception of pheromones have received little study, but C. Masson (CNRS, Marseilles) has developed promising techniques for recording olfactory responses from the antennal lobe of the ant brain.

The deeper understanding of insect behaviour gained from studies on pheromones is opening up new avenues for pest control. F. J. Ritter, A. M. van Osten and C. J. Persoons (TNO, Delft) reported the discovery in fungus-infected wood of terpenoid substances which may be identical with the trail pheromone of the termite *Reticulitermes santonensis*. Related compounds from sandalwood oil are also very effective in inducing trail following. Such substances could be used to divert members of a colony to an area where they could be contaminated with slow-acting insecticide. On return to the nest they would contaminate their nest mates. J. M. Cherrett and his colleagues (University College of North Wales, Bangor, and Rothamsted Experimental Station) have found that the trail pheromone of the leaf-cutting ant, *Atta texana*, markedly increases the effectiveness of baits used for controlling several species of leaf cutters, and that naturally occurring arrestants and phagostimulants can also enhance the properties of the baits.

Insect juvenile hormone (JH) and its analogues can now be obtained com-

Release of Messenger RNA from Nuclei

It is known that the release of messenger and ribosomal RNA from nuclei *in vitro* is dependent on a supply of energy and on non-dialysable components from the cytosol. The results of experiments reported by Schumm *et al.* in *Nature New Biology* next Wednesday (October 17) define further the nature of the non-dialysable component.

Schumm *et al.* have found that an ammonium sulphate fraction (33–66%), representing a 50% yield of the total cytosol activity, subjected to DEAE cellulose fractionation and freeze thawing, results in a five- to ten-fold increase in transport of messenger RNA out of the nuclei of liver cells incubated *in vitro*. The molecular weight of the purified cytosol fraction was inferred to be between 10,000 and 30,000, and it is im-

plied that the active substance is a protein.

Hybridisation studies show that the purified cytosol factors impose some specificity on the RNA released from the nuclei. The sequence homology between three closely related cell types, normal, 19-hour-regenerating and neoplastic liver, was less than 50% when the heterologous, as compared with homologous, purified cytosol factor was used. The authors themselves point out the limitations of the hybridisation technique employed, however, which is such that only highly redundant nucleic acid sequences are detected. The system would seem to offer possibilities for the study of the mechanism of RNA processing in and of RNA release from the nucleus.

mercially, and many experimenters have taken advantage of this to study its role in caste determination. K. Wanyoni and M. Lüscher (University of Berne, and ICIPE, Nairobi) found that JH analogues can prevent the formation of reproductives in orphaned colonies of the termite *Zootermopsis*. With his colleague D. Meyer, Lüscher found JH activity in the anal secretion of the queen of the mound-building termite, *Macrotermes subhyalinus*. This work gives a further pointer to the involvement of JH in termite caste regulation. I. Hrdý (Czechoslovak Academy of Sciences, Prague) induced mortality and abnormally high soldier production on treating termites with JH analogues. The application of these substances to honeybee colonies resulted in death of some of the brood and sometimes of the queens. There is thus a possibility that some JH analogues could be used in insect control if their action against honeybees can be restricted.

Although few contributions were concerned with taxonomy, some of the behavioural and ultrastructural investigations that were reported shed light on phylogenetic relationships. H. Markl (Technische Hochschule, Darmstadt) surveyed the occurrence of stridulatory organs in ants and related this to their nesting ecology. Stridulatory organs are found in ground-nesting species and are usually lost when species become surface-nesting or arboreal. R. Koltermann (University of Frankfurt) compared the ability of the Indian honeybee, *Apis cerana*, and three races of *Apis mellifica* to learn an association between food and scent. There were marked differences between the two species and among the three subspecies in their ability to 'remember' different scents, for example, the Indian bee remembered orange oil, relative to other scents, better than the Egyptian honeybee, and the Egyptian bee remembered orange scent better than did the Italian bee. This variation in associative learning ability correlates with the distribution of the orange tree, which originated in Asia and spread to the Mediterranean area in the sixteenth century, but is not found in central Europe.

Copies of the proceedings of the congress (seventy published papers) can be obtained from Dr P. E. Howse, Biology Department, University of Southampton (£3.25, post free). An important development for the future of IUSSI was a revision of the constitution made at the congress providing for a permanent secretary-general (Dr Howse) whose main duty will be to collect and distribute information among those working on social insects. This should facilitate international cooperation among entomologists and also help to avoid duplication, especially where work in the tropics is concerned.

RADIATION

Safe Doses

from a Correspondent

THE current preoccupation of the people of the United States with consumer pressure groups would have been an important topic at the Third International Congress of the International Radiation Protection Association even if it had not been held in Washington DC on September 9-14. The public reaction in the United States to the application of nuclear energy for electric power production and the associated cost of protecting the environment is of widespread interest, especially when contrasted with the demand for the reduction in the population dose from the use of X rays for medical diagnosis. G. H. Whipple (University of Michigan) debated the intention of the International Commission on Radiological Protection in recommending that radiation doses should be kept "as low as practicable". He accepted this as a qualitative admonition to prudent judgment and discussed the US Atomic Energy Commission's recent adoption of this as a regulatory standard with numerical limits. He claimed that the AEC philosophy demanded "if it can be done then it must be done" and was without regard for economic and social cost. It was suggested that the application of this philosophy could increase the radiation exposure of reactor operational staff. R. Lapp (consultant, USA) further alleged that the greatest benefit to the public of the United States would come from tackling medical diagnostic X-ray exposure and this was where

public expenditure was really required.

B. Lindell (Swedish National Institute for Radiation Protection, Stockholm) offered a simple approach to the allocation of public funds based on the thesis that the dollar equivalent of 1 rad dose to 1 man was \$100. The investment of \$60 to reduce the dose to a female from a urography X-radiation examination would be justified if it saved 0.6 rad dose. He suggested that it would be appropriate to invest vast sums to reduce avoidable exposure to the whole world population even if this came from natural radiation. The estimated radiation cost of the nuclear power programme was modest, especially when compared with the world population dose from atomic bomb testing.

An estimate of worldwide doses from carbon-14 produced by nuclear reactors was given by V. P. Rublevskiy (Biophysics Institute of the USSR Health Ministry). His prediction of a ten-fold increase in carbon-14 concentrations in the biosphere by the year 2010 implies that some control on these releases may be necessary.

This congress was a timely opportunity for a presentation on the Report of the Advisory Committee on Biological Effects of Ionising Radiations (National Academy of Sciences, Washington, 1972) led by C. L. Colmar (Cornell University). This report was written to provide the United States with a basis for standard setting and gives numerical estimates of the risks from ionising radiation exposure. The authors chose to base their calculations on the assumption that the whole of the US population would eventually receive

Spacing of Histones on Chromatin

In *Nature New Biology* next Wednesday (October 17) Bustin presents a fresh approach to the study of the arrangement of histones on chromatin. Isolated, fractionated, histones are antigenic, but their specific antibodies do not react with chromatin. What Bustin has discovered, and put to good use, is that isolated, fractionated, histones when bound, individually, to RNA will elicit antibodies which will react specifically with the appropriate histones within the native complex.

From this interesting observation, Bustin concludes that the antigenic determinants in free histones differ from those of complexed histones. This is not surprising in that previous physicochemical studies on the isolated histone fractions have shown them to be conformationally very different (more open) in comparison with the same histones *in situ*. That Bustin attempted the approach described in his forthcoming communication does not seem to have been suggested to him by findings al-

ready in the literature, which attests to its originality.

Several fascinating conclusions have emerged from the study of the interaction of one or more of the histone (RNA)-specific antibodies with the corresponding native chromatin: (1) In native chromatin the antigenic determinants of F₁ and F_{2b} are more available to interact with homologous antibody than those in F₃ and F_{3a1} and the determinants of F_{3a2} are least available. Bustin suggests that the availability of the various determinants is probably dependent on steric factors (for example, burial of histones inside the complex) and/or conformational changes that occur in the histones on interaction with DNA and other chromosomal proteins. (2) Serial binding of up to four different specific antibodies results in incremental binding. This result suggests that the various histones are spatially separated enough so that their determinants do not overlap. Further ramifications of Bustin's new approach are on their way.

the maximum dose permitted in current guides. Their calculation of up to 27,000 cases of genetic defects or diseases per year should be viewed in the light of estimates that the US population dose is unlikely to exceed 1% of the current guide levels.

The International Commission on Radiation Units and Measurements (ICRU) was represented by its Chairman, H. O. Wyckoff, who announced their intention to retain a special unit (the rem) for the quantity of Dose Equivalent. In spite of the temptation of the *Système International* and in the face of the logic of those who regard the unit as an expression of risk, the ICRU will retain the rem as an aid to clear and safe communication. W. S. Snyder (Oak Ridge National Laboratory) gave advance warning of ICRP changes in internal radiation dose concepts and said that the Maximum Permissible Annual Intake would be used in future as a quantity directly related to the annual dose received. The appropriate ICRP reports are being re-issued and these will give derived limits for occupational exposure to airborne radioactive contamination.

The meaning of the congress and association title is now taken to embrace non-ionising radiation. This topic was reviewed by H. Jammet (Commissariat à l'Énergie Atomique, France) who discussed the hazards of microwave and ultraviolet radiation as well as the potential damage caused by laser beams. He was able to quote some safe exposure levels for these radiations but had found no equivalent to the ICRP and reported that there is a lack of satisfactory radiation monitoring systems and in some cases biological data are inadequate.

FOREST ECOLOGY

Nutrient Cycles

from our Plant Ecology Correspondent

SURPRISINGLY there is little reliable information regarding the rate at which various chemical elements pass through ecosystems. Such data are particularly valuable, since the pattern and rate of nutrient cycling may well be related to the productivity and the stability of some ecosystems (see, for example, Odum, *Science*, **164**, 262; 1969). It is good to know both the overall budget of input and output of nutrients to and from a system as well as the pattern and rate of movement of nutrients within the system.

The Hubbard Brook ecosystem study in the northeastern United States has attempted to produce this information for temperate deciduous forest. Most of the information which has emerged so far from this project has been concerned with overall nutrient budgets for

such elements as calcium, potassium (*Ecology*, **48**, 772; 1967), and the way in which such budgets are modified by experimental perturbation of the system by felling and herbicide treatment (*Ecol. Monogr.*, **40**, 23; 1970). The most recent paper from the Hubbard Brook study is by Eaton, Likens and Bormann (*J. Ecol.*, **61**, 495; 1973) and is concerned with nutrient movement within the forest ecosystem, particularly between the forest canopy and the soil.

Eaton *et al.* describe the leaching of a canopy by rainfall, a process which transfers soluble material directly from the leaves and branches to the soil without the intervention of decomposer organisms. This leaching is considered as the sum of throughfall (water passing directly through the leaf canopy to the forest floor) and stemflow (water running down branches and trunks). Stemflow was found to contain a higher concentration of nutrients than throughfall, but only 5% of the total precipitation followed this path, hence its contribution to the overall leaching was small.

The total quantities of elements leached from the canopy varied from one element to another, in some cases exceeding the quantities arriving at the forest floor by litter fall. For example, potassium was leached at a rate of 30 kg ha⁻¹ between June and October, whereas annual litter fall resulted in the movement of only 21 kg ha⁻¹ of potassium from canopy to floor. The

figures for sulphate sulphur are similar, 21 kg ha⁻¹ being leached during the study period, only 6 kg ha⁻¹ yr⁻¹ arriving at the soil surface by litter fall.

The elements quoted are usually present in ionic form, which may account for their rapid movement in solution. Elements normally occurring bound in organic molecules within plant tissues were found to be leached far more slowly. In the case of total nitrogen, 10 kg ha⁻¹ was leached from the canopy during the summer and a total of 56 kg ha⁻¹ arrived on the forest floor as litter during the year. Similarly for phosphorus (as PO₄³⁻), 0.68 kg ha⁻¹ was leached and 3.7 kg ha⁻¹ yr⁻¹ fell as litter.

In essence, these figures resemble those which Carlisle, Brown and White (*J. Ecol.*, **54**, 87; 1966) obtained from temperate deciduous forest in Britain. Eaton *et al.* go further than these workers in that they demonstrate the importance of hydrogen ion exchange as a mechanism in leaching cations from the canopy. The pH of precipitation at Hubbard Brook has a mean of 4.06; throughfall pH was 5.01. Hydrogen ions thus represented 74% of the cation input and only 2% of the cation loss. At least 27% of the total cation loss can be accounted for by hydrogen ion exchange. The low pH of the precipitation at Hubbard Brook may well derive from atmospheric pollutants since it cannot be accounted for by equilibration with atmospheric CO₂. If this is so,

Formation of Cosmic Formaldehyde

THE discovery of the first organic molecules in space, nearly five years ago, took most astronomers by surprise because it seemed unlikely that molecules could form and survive in the harsh environment of interstellar space. But there are now twenty-six molecular species identified with reasonable certainty, and the question of the creation of these molecules has assumed some importance.

In the burgeoning subject of astrochemistry, Dalgarno and his colleagues at Harvard College Observatory have sketched some of the pathways by which the simpler molecules can be synthesised. Three months ago they pointed out (*Astrophys. J. Lett.*, **183**, L21; 1973) that positive and negative ions in interstellar clouds play an important part in the production of complex molecules. For example, the chemi-ionisation reaction $\text{CH} + \text{O} \rightarrow \text{HCO}^+ + e$ is an important source of ions and electrons in cosmic conditions. The presence of HCO⁺ can then lead to the formation of more exotic species.

In *Nature Physical Science* next Monday (October 15) this work is extended in a discussion of the formation of formaldehyde by Dalgarno, Oppen-

heimer and Black. This molecule is one of the most widely distributed and abundant species in the Galaxy. In dense interstellar clouds one possibility is to build H₂CO from HCO⁺. Dalgarno and his colleagues suggest, however, that the reaction $\text{CH}_3 + \text{O} \rightarrow \text{H}_2\text{CO} + \text{H}$ would be more likely as a source of formaldehyde in the diffuse interstellar clouds. In this picture the CH₃ is built up in a three-stage process: photoionisation of carbon by starlight gives C⁺; this is followed by $\text{C}^+ + \text{H}_2 \rightarrow \text{CH}_2^+ + \text{h}$, and finally $\text{CH}_2^+ + \text{H}_2 \rightarrow \text{CH}_3^+ + \text{H}$.

Although the formaldehyde itself is vulnerable to destruction by photoionisation, the authors show that for a wide range of densities and ultraviolet fluxes, the formation rate exceeds the destruction rate. A further source of formaldehyde is the reaction $\text{CH}_3^+ + \text{O} \rightarrow \text{H}_2\text{CO}^+ + \text{H}$; the H₂CO⁺ ion then loses its positive charge through exchange with heavy elements to leave formaldehyde.

With the appearance of the results of Dalgarno *et al.* there seems to be no fundamental difficulty in accounting for the widespread presence of formaldehyde in the Galaxy.

it provides one more example of the way in which human activities tend to result in the acceleration of natural nutrient cycles.

EARTH'S MANTLE

Non-Newtonian Flow

from our Geomagnetism Correspondent

ALTHOUGH the precise nature of the force driving the drifting continents and spreading ocean floors is still a matter of dispute, it is generally agreed that the drifting and spreading processes involve large-scale flow in the Earth's upper mantle and possibly in the lower mantle as well. But what sort of flow? Most quantitative models of mantle behaviour have been based on the assumption that flow is Newtonian; that is, that the mantle obeys the laws which characterise Newtonian fluids. Post and Griggs (*Science*, **181**, 1242; 1973), however, have now challenged this view, basing their case for non-Newtonian flow in the mantle on both field observations and laboratory experiments.

Interestingly, the field observations are those relating to the isostatic uplift of Fennoscandia—the classic phenomenon used in the earliest attempts to determine the mantle's viscosity. When the glacial ice receded from Fennoscandia about 10,000 years ago, the remaining surface depression was partly removed by immediate elastic rebound. Since then, however, there has been continuous isostatic adjustment during which the shape of the depression has remained roughly constant and for which the current rate of uplift is everywhere proportional to the magnitude of the depression. Post and Griggs believe that these properties indicate a special type of behaviour which they term 'proportional relaxation' and in which the ratio of the effective shear stresses at any two points remains constant with time while the stress amplitudes decrease asymptotically to zero.

The validity of proportional relaxation as defined by Post and Griggs rests on the assumption that the mantle is incompressible. In this case, the stress distribution in the mantle will be consistent with the surface depression and the total viscous dissipation will always be at a minimum. As long as the shape of the stress distribution in the mantle depends on the shape of the surface depression but not on its magnitude, it then follows that the viscous dissipation will remain at a minimum if the surface elevations and the original stresses are multiplied by the same factor. Thus if the shape of the surface depression does not change with time (as in Fennoscandia), nor will the shape of the stress distribution. In short, there is proportional relaxation.

The general form of the law of flow

in the mantle chosen by Post and Griggs is $\dot{\epsilon}_s = B(z)\tau^n$, where $\dot{\epsilon}_s$ is the steady state effective strain rate, B depends only on depth (z), τ is the effective shear stress, and n is a constant. For Newtonian flow n would be 1 and, as can be shown from the general flow law, the logarithm of the remaining uplift at the centre of the uplifting area would vary linearly with time. But from the remaining uplift in Fennoscandia, Post and Griggs show there is no such linearity, and n is 2.85, 3.21 or 3.58, respectively, for the three published estimates of the remaining Fennoscandian uplift (150 m, 180 m and 210 m). In other words, as long as the proportional relaxation model is valid (which requires, among other things, that n be independent of stress), postglacial uplift in Fennoscandia requires non-Newtonian flow in the mantle with an n value of 3.2 ± 0.3 .

The second part of the case rests on deformation experiments with dunite, whose chief constituent, olivine, is generally thought to be an important constituent of the upper mantle. Creep and high temperature relaxation experiments on dunite from Mt Burnett show that the best-fitting value of n is 3.18 ± 0.18 . This is in excellent agreement with the n value obtained from uplift and is thus consistent with the idea of non-Newtonian mantle behav-

iour. In view of the large number of assumptions used in the reanalysis of Fennoscandian uplift, Post and Griggs would regard such close numerical agreement as fortuitous. But whatever the precise figure, the flow certainly seems to be non-Newtonian; and plate tectonic models involving mantle motion should incorporate it.

LOW TEMPERATURE PHYSICS

Superconducting Bridges

from our Condensed Matter Correspondent

THE interesting structure which appears in the current-voltage characteristics of very small superconducting bridges at subharmonic values of the energy gap can be attributed to the effects of Josephson radiation generated in the bridge itself, according to Gregers-Hansen, Hendricks and Levinson, of the University of Copenhagen, and Pickett, of the University of Lancaster (*Phys. Rev. Lett.*, **31**, 524; 1973).

The Josephson effect is a phenomenon which occurs when two regions of superconductor are coupled together through a very weak link. The link can either be a short narrow bridge made of the same material, as in the present case, or it can be an exceedingly thin layer of insulator which electrons can traverse

Glaciation and the Chalky Boulder Clay

MANY years ago, Harmer (*Jub. Vol. geol. Ass.*, **103**; 1909; and *Proc. Yorks. geol. Soc.*, **21**, 79; 1928) concluded that the distribution of Chalky Boulder Clay in Britain was brought about by two distinct ice streams. The first passed from the North Sea to parts of the Lincolnshire Wolds and was then deflected southwards, fanning out into East Anglia and the Midlands; the second entered across the present north coast of Norfolk and came into contact with the North Sea Drift. The boundary between the two deposits has long been in dispute, but, even more fundamentally, there has been disagreement over the number of glaciations involved. Harmer believed that both streams were of the same glaciation; but more detailed studies carried out during the late 1940s and early 1950s apparently suggested that two glaciations were concerned—the lower Lowestoft and the upper Gipping. The second view then prevailed until the late 1960s when the controversy was revived.

In *Nature Physical Science* next Monday (October 15), Perrin *et al.* report a detailed study which suggests that, in fact, the Chalky Boulder Clay represents only one glaciation. Samples of Boulder Clay from eleven sites previously ascribed to the Lowestoft glaciation and twelve sites ascribed to the Gipping

glaciation have been analysed for mechanical composition, calcium carbonate content, heavy mineral percentages and clay mineral content. As far as mechanical composition is concerned, no systematic differences are observed between Lowestoft and Gipping samples. Calcium carbonate content is more variable, but the trends are consistent with gradual assimilation of chalk by ice moving in the directions described by Harmer. Nor are any significant variations observed in heavy mineral percentages and clay mineral content. In view of the compositional consistency over a wide area (14,000 km²), Perrin and his colleagues conclude that the involvement of two glaciations cannot be substantiated. Moreover, the one glaciation concerned is the Lowestoft; there is no evidence for the effects of a later Gipping glaciation.

As Perrin *et al.* point out, however, even if the Chalky Boulder Clay represents a single glaciation, its origin remains in doubt. The oft-stated assumption that much of the matrix was derived from the Jurassic clays must be ruled out on compositional grounds; and the virtual absence of Triassic or Pennine debris militates against westerly or north-westerly sources. For the time being, a North Sea origin still seems the most likely.

by quantum mechanical tunnelling, as for superconducting tunnel junctions. In either case, a current flows which consists of an a.c. component of frequency $2eV/h$ where e is the electronic charge, V the voltage and h is Planck's constant; and, under appropriate conditions, a d.c. component is also present. The device therefore generates electromagnetic radiation, whose frequency $2eV/h$ depends on the applied voltage, known as Josephson radiation.

The superconducting microbridges developed by the Copenhagen group are in the form of very thin metal films prepared by an ingenious technique on glass microscope slides. The slide is first scratched very lightly with a razor blade, and then etched briefly in dilute hydrofluoric acid, producing a uniform groove 5×10^{-7} m wide and of semi-circular cross section. A layer of tin or indium about 10^{-7} m thick is then deposited on the slide in an evaporator, and this film is divided into two parts by a second razor blade cut, at right angles to the groove. The metal deposited at the bottom of the groove, however, is protected from the final cut and so forms a bridge between the two halves of the divided film. Such microbridges can be made, typically, 5×10^{-7} m wide and 2×10^{-7} m long, which is considerably smaller than has been achieved by other workers using more conventional techniques.

Plots of current i against V for these devices showed, among other interesting effects, a series of very slight kinks when eV was less than the energy gap, 2Δ , which separated the superconducting and normal electrons. By measuring dV/di rather than i , a series of sharp peaks was obtained; each peak occurred when V was approximately $2\Delta/Ne$, where N is an integer. Because a peak in dV/di corresponded to a reduction in i , it was necessary to explain why the current should be suppressed at these particular values of V .

Gregers-Hansen *et al.* suggest that the phenomena were caused by the Josephson radiation, given out by the bridge, acting on itself. They point out that, in metal microbridges, harmonics of the radiation, at frequencies $2NeV/h$, are always also present. Whenever V is such that the energy $2NeV$ of a photon is equal to 2Δ , there will be a probability of that photon being absorbed in exciting a pair of superconducting electrons across the energy gap into normal states. Thus, whenever $2NeV = 2\Delta$, some further suppression of the superconductivity of the bridge, and a corresponding drop in the current, are to be expected, in agreement with the experiments.

This elegant explanation of the phenomena runs into one apparent snag: the peaks in dV/di do not, in fact, occur in quite the right places.

Gregers-Hansen *et al.* evade this difficulty, however, by noting an earlier theoretical prediction by Eliashberg (*J.E.T.P. Lett.*, **11**, 114; 1970) that Δ should be increased by the application of a microwave field. They suggest that the energy gap in the bridge is being changed by its own Josephson radiation and, acting on this assumption, have analysed their data to determine Δ as a function of V .

As a check on this interpretation of the measurements, they repeated the experiment in an externally applied 9 GHz microwave field. One effect was that each dV/di peak acquired sidebands, exactly as would be expected if the Josephson radiation were mixing with the external microwave field to give the sum and difference frequencies: this is convincing evidence in support of their ideas.

Similar subharmonic structure has frequently been observed in tunnel junctions, and several very elaborate and apparently successful theories have been devised to account for it. It now seems clear from the Copenhagen results that these phenomena arise, in reality, from small metallic short circuits in imperfect junctions.

NUCLEAR STRUCTURE

Heavy Ions

from a Correspondent

THE increasing interest in the use of accelerated beams of heavy ions was highlighted at a nuclear structure conference sponsored by the Institute of Physics and held at the University of Manchester from September 5 to 7.

The contribution made to the understanding of the structure of the nucleus through the study of single nucleon transfer reactions induced by light ions has been considerable. Now with the increasing availability of heavy ion beams of good energy resolution and with advances in experimental techniques and improvements in the understanding of the reaction mechanism, the study of transfer reactions induced by heavy ions promises to extend the usefulness of this spectroscopic tool. G. C. Morrison (University of Birmingham) reviewed the progress which has been made in this exciting field during the past few years. From studies of single nucleon transfer much has been learned about the mechanism of the heavy ion-induced transfer reaction and the importance of angular momentum selection rules. It is, however, in the study of multinucleon transfer reactions that valuable new spectroscopic information is to be expected and several examples of such reaction studies were reported at the meeting.

M. A. Grace (University of Oxford)

described the very elegant experiments being carried out at Oxford to measure the g factors of excited nuclear states. The technique involves measurements of the Larmor precession of the nuclear moment in the hyperfine field of a hydrogen-like ion. For example, the $H(^{19}F, \alpha)^{16}O^*$ reaction has been used at a fluorine bombarding energy of 48 MeV to measure the g factor of the 3^- state of ^{16}O . As a result of this heavy ion-induced reaction the oxygen ion recoils into vacuum with a simple electronic configuration, namely one electron in the $1s$ shell. In these circumstances the magnetic field acting on the nuclear moment is calculable (86 Mgauss) and thus a direct measurement of the frequency of Larmor precession yields the magnetic moment of the state.

The anomalous behaviour of high angular momentum states of rotational bands in the rare earth region, predicted in 1960 by Mottelson and Valatin and first observed experimentally as recently as 1971, continues to be the subject of much experimental and theoretical interest. Thus in a review of recent theoretical developments in the study of heavy nuclei K. Kumar (Vanderbilt University, Nashville, and Research Institute for Physics, Stockholm) described the successes of the pairing plus quadrupole model and in particular demonstrated that the observed features of the anomalous behaviour of the energy levels at high angular momentum could be understood within the context of this model.

I. S. Grant (University of Manchester) discussed the important parts played in the fission process by pairing and shell effects. The recent identification of a rotational band in the second potential minimum and the observation of γ -ray decay from the second well to the first have provided new information on the shape of the nuclear energy surface at large deformation. Theoretical attempts to explain the deformation of the second potential minimum have, encouragingly, been rather successful. The use of heavy ion projectiles also allows the role of angular momentum to be investigated in the formation of a compound nucleus and in the fission process. One interesting new advance here is the evidence for the enormous importance of viscosity in the fusion of very heavy ions and nuclei.

Other invited talks were given by T. S. Green (Culham Laboratory, Abingdon) who discussed plasma sources for heavy ion production, G. Astner (Research Institute for Physics, Stockholm) who reviewed the present state of understanding of effective electric quadrupole charges, and R. Huby (University of Liverpool) who described the impressive theoretical advances which have been made in the description of stripping to unbound states.

Science as Intellectual Entertainment

NIGEL CALDER

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A science writer who has been closely involved in the creation of *The Life Game*, which is to be shown on BBC Television tomorrow night, discusses the aims of epic science programmes on television and some of the difficulties encountered.

"THE generality of mankind cannot accompany us for one short hour unless the path is strewn with flowers", Michael Faraday once remarked. During the past few years, the British Broadcasting Corporation and a growing consortium of overseas partners have experimented with 'big' television programmes on major scientific themes as a means of reporting to the public from the very frontiers of research: *The Violent Universe* (1969, 150 min), *The Mind of Man* (1970, 120 min) and *The Restless Earth* (1972, 120 min). The latest of them, *The Life Game* (150 min), deals with evolution and related topics in biology and has been made by the BBC as a coproduction with television organisations in seven other countries. Altogether seven versions of the programme are being seen by their respective audiences, with the same story told in different languages and accents. In this article I shall outline, as best I can, the process by which we decided what the story should be.

Means and Ends

Roughly a year is allotted to making one programme and the budget, although not of Hollywood proportions, is substantial for a documentary. About two dozen sequences can be filmed around the world, showing scientists at work; in some cases the scientists address the camera, but they have to be concise and forceful. A large BBC television studio is booked for a week and the overseas coproducers send their presenters to London to use the elaborate sets that have been prepared, for recording the studio links. These are eventually interspersed between longer passages on film.

For the scientists taking part as well as for the viewers there is a sense of occasion: the theme is to be treated generously, in a way worthy of its importance, bringing together ideas that are otherwise left fragmentary and unconnected. The aim is intellectual entertainment. We do not think of such programmes as 'educational' in any narrow sense of the word: no one is going to have to sit an examination in the subject and, if each viewer is stirred by it in his own way, all well and good. A properly constructed programme should have something to say at several different levels, depending on the prior ignorance or knowledge of the viewer. Nevertheless, we set out to report in plain language at least a sample of the new findings, ideas and issues that would be under discussion if there happened to be a high-level gathering of experts in the subject, at about the time the programme is transmitted.

Communicating to the public from the frontier is actually easier than from the rearward areas of long-established knowledge. For one thing the questions are starker: What is a quasar? How does the brain learn? Why do mountains exist? How does an animal grow? They are the sorts of questions that a layman, if need be with a little prompting, would ask himself. To the extent that the scientist is airing his doubts and ignorance, the viewer feels less separated from him by a

wall of expertise. The jargon has not been invented yet, nor have the textbooks been written that impose a formal way of presenting knowledge from which mental liberation is often difficult. At the frontier, too, the scientists themselves are excited and that enlivens the whole programme in a way that cannot be simulated for older discoveries. And one is showing science in something like its true colours—as a process of discovery rather than a mass of schoolroom facts.

Of course, the script must repeatedly dip into the older knowledge for basic information but the notion that new knowledge is more difficult than old is a fallacy that clogs our culture. In *The Violent Universe*, we showed unpatronisingly—rather dramatically in fact—that the Sun is an ordinary star. That turned out to be news for some of our viewers, and for them, at least, the proposition that a pulsar is a neutron star must have seemed plain sailing by comparison. Advances like plate tectonics can suddenly make transparent sense of whole libraries of previously inexplicable facts. In *The Restless Earth* we sought to capture that moment of human enlightenment.

Preliminaries

The idea for a two-hour biology programme began to germinate while the work on *The Restless Earth* was coming to an end. The special problem, though, stood out right away. A basic assumption was that, for any programme of this kind, the subject should be a big one—grandiose even—and of such obvious importance and interest that families would be prepared to give up an evening to it. After the Universe, the brain and the Earth, nothing less than 'life', treated in a broad way, would do as a theme. Yet life was altogether too elaborate both in its natural complexity and in the enormous diversity of specialists pursuing it—from protein chemists and immunologists to ethologists and palaeontologists. The biology of *E. coli* could easily fill two hours, but how would that be billed in the *Radio Times*?

The problem, therefore, was to find a cluster of topics in current biology, of reasonable coherence, plainly related to life as perceived by the ordinary viewer but also reflecting a good deal of the present excitement in fundamental biology. The first and obvious step towards a solution was to take evolution as the great unifying theme of biology. Evolutionary research was in a lively state and molecular biology had taken its rightful place in it as well as having a near monopoly in respect of the origin of life. The chief complication was developmental biology, which many would regard as the cutting edge of present research into life. About its fascination there was no doubt—but could it be fitted into an evolutionary framework in a better than Procrustean fashion? At any rate we had, in June 1972, a simple checklist to start with: evolution theory; relevant molecular biology; origin of life; developmental biology (?). But that had to be translated into a collection of specific locations, experiments, propositions, contributing scientists—in short, into filmable sequences. And those sequences would have to fit together as passages in a single story.

For four years both Philip Daly, then the producer, and I had been too busy masquerading as astronomers, brain researchers and geologists to keep more than the unsteady eye on fundamental biology. It is shaming to admit it now, but the first time Motoo Kimura's name came up I had to ask how to spell it—yet he launched his great evolutionary heresy

of the neutral genes in *Nature* in 1968. The very work of learning, over a period of months, and personal feelings of surprise or fascination or confusion as new aspects of the story unfold, are part of the explanation of how one decides what to tell the public.

A reconnaissance, carried out with as few preconceptions as possible, has been the key to the method of working. The producer and writer undertake a world tour, without cameras, just to visit 100 to 200 specialists in perhaps a dozen countries, even before the scope of the programme is defined. We begin by consulting a few leading authorities; a list of possible topics and experts grows almost exponentially at first, but quickly stabilises as the same names and subjects begin to recur. Soon there is a basis for planning the tour, though one must check to see whether important topics or viewpoints are being overlooked. Finding out about science by word of mouth from the practitioners reveals trains of thought, interconnections and ambitions that are rarely explicit in the formal literature. Most of those who are generous with their time and advice, on which we wholly depend, do not in the end figure in the programme, but everyone contributes to the perspective, which is most important of all.

During the reconnaissance, the producer is assessing the intrinsic fascination of each topic or experiment and considering what use he could make of it visually. As we are working in scientific 'real time', we have to ask at what stage the work will be when filming is in progress and whether new results will be published before the transmission date. The writer is absorbing as much information as possible while trying at the same time to stand back and see how the topic under discussion links with other parts of the story. First the content and then the shape of the programme emerge from continuous debate between producer and writer.

Some cardinal topics in evolutionary biology were adopted as 'seeds' on which other constituents of the programme might crystallise: protein polymorphism, for example, and the strange changes in functional molecules in the course of evolution. Both of these discoveries contribute to Kimura's argument in favour of nearly neutral genes. Despairing of any resolution of the neutralist-selectionist controversy within the year of the programme, we set Kimura and his most distinguished opponent, Theodosius Dobzhansky, against one another. The explosive evolution among the *Drosophila* of Hawaii provided an irresistible example of current investigations into the origin of species. As for human evolutionary genetics, besides the already classic elucidation of sickle-cell anaemia, there was newer material in, for example, investigations of the genetic consequences of the spread of Neolithic agriculture.

Sometimes we were looking for new ways of dealing with well publicised themes. Prebiotic synthesis of organic materials was a case in point, and we turned to the research on the Martian atmosphere as a potential source of such compounds.

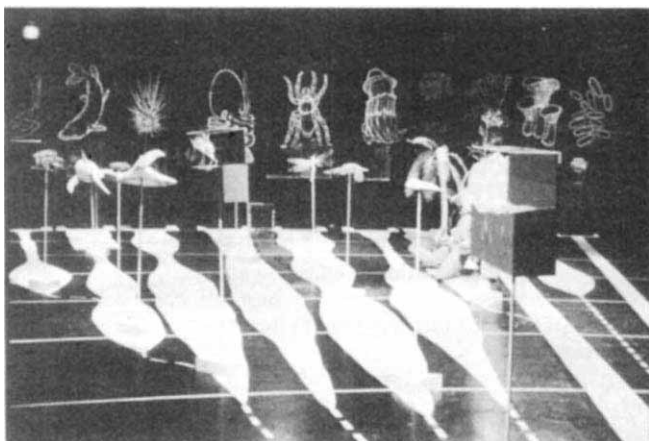


Fig. 1 A BBC Television studio was used to display evolutionary relationships. The British Museum (Natural History) gave advice about the exhibits. (BBC copyright.)

We also needed chronological and conceptual bridges from the origin of life to the Phanerozoic Era. We found them in work on early microfossils, in the symbiotic theory of the origin of eukaryotic cells, and in experiments on the origin of enzymes.

Games

Quite early on in the reconnaissance the 'game' theme began to obtrude. The metaphor of life as a game, in which the effects of chance are controlled by rules, had been in our minds from the outset. More substantial uses of games appeared unbidden in the laboratories that we visited, including the application of the theory of games to fighting behaviour in animals and the Monte Carlo method used in genetic computations. In Göttingen, Manfred Eigen had a real game for us, played by contestants around a table, who evolved cloverleaf RNA molecules by substituting bases according to the throw of a specially made tetrahedral die.

As for fitting developmental biology into the evolutionary context, a ready-made solution offered itself. The model that involves the repetitive DNA in the control of genes in higher organisms, developed by Roy Britten and Eric Davidson in the USA, was looking good, experimentally. These investigators had extended their model as an explanation of how, in the course of evolution, novel structures and functions might appear in animals. Given this link—which, as we verified, is taken seriously by leading systematists as well as by molecular biologists—we could relate several striking experiments in developmental biology to our main theme.

The reconnaissance brought unexpected bonuses. Richard Leakey, when we first met him, was just about to announce the discovery of the remarkable hominid skull 1470 from East Rudolf. In the Galapagos Islands, our guide was the British ornithologist Michael Harris. Although, in the end, the Galapagos were to disappear from the programme, we learned that Harris had done recent experiments in Wales on the classic circular overlap of the sympatric species of gulls, *Larus argentatus* and *L. fuscus*. On the other hand, topics carefully considered for *The Life Game* but omitted included cell fusion, hormones in insect development, the molecular biology of viruses, genetic effects of radiation, the Orgel-Crick tongue-in-cheek hypothesis of directed panspermia, and social behaviour in lemurs. This last we had investigated to the extent of creeping around the forest in Madagascar looking for *Indri indri*.

Even this partial list of omissions shows the unwieldiness of the subject we were tackling, and at the same time as our notebooks were filling with increasingly heterogeneous material we were battling to find a pattern. The logic of a television programme is quite different from formal instruction in science. Apart from the great inversion already mentioned (starting at the frontier and working back as necessary) the sequence and arrangement of topics depend at least as much upon anticipation of questions arising in the viewer's mind, and upon dramatic considerations, as upon what the scientist would regard as the logical flow of ideas. One has to think especially of the rise, through each group of sequences, to some kind of climax. Some topics are 'starters', in the sense that they have to be followed by something else to justify them; others are 'finishers', after which to carry on their particular lines of thought would be anticlimactic. Decisions about how to open the programme and how to finish it are paramount, because here are the interfaces with the world of teacups and family chatter.

The question of whether an important point may be difficult to explain does not affect the choice of topics unduly. Where exposition becomes tricky, one turns to radical methods, such as the drainable topographic model of the North Atlantic used in *The Restless Earth* to explain ridges and trenches. For *The Life Game* we recruited a hundred Swedish children to perform a 'dance of the molecules', in order to illustrate protein synthesis, mutation and the like, as painlessly as pos-

sible. Such resorts are part of the task of 'strewing the path with flowers'.

The shooting script sets out all the sequences that are to be filmed in a possible running order. It is a guide to the film director (normally the producer) and to the scientists concerned as to the minimum needed on film for the scientific exposition. In practice, the director uses his own creative imagination in portraying the work, and it is this which makes the difference between a visually rich documentary programme and a pedestrian instructional film. The shooting script serves an important supplementary function. Copies of the complete script are sent to several leaders in the science, for comment as to the overall content, balance and argument, while each scientist whose work is being filmed sees the relevant section of the script and he, too, can criticise the approach adopted and correct the draft commentary as necessary. But the script has also to be judged as television and for that purpose it goes to the science producers of the coproducing organisations, for criticisms and suggestions.

At this stage, for *The Life Game*, our procedures were put to a severe test when Philip Daly was promoted and a new producer took charge. To Adrian Malone, fresh from years of work on his series *The Ascent of Man*, fell the task of building the programme without the benefit of the reconnaissance. I must pass over the months of effort that Malone and his colleagues put into the filming: the arduous travel, the difficulties with the weather, the patient work of setting up shots, and the tolerance of the scientists whose laboratories were invaded. The filming provided raw material for the programme, but it was in the editing that it began to take final shape.

Putting Together

There was an inevitable casualty list of sequences that for one reason or another were not obtained, or turned out unsatisfactorily. In other cases far more time proved necessary for setting a scene or explaining a process than the script originally envisaged. In the end, several sequences had to be omitted from *The Life Game*, even though the duration of the programme was extended from 120 to 150 min. Whole trains of thought were shunted out of the show and this painful process entailed repeated rethinking of logic and balance.

The final running order was quite different from that envisaged in the shooting script. Everyone's ideas had become sharper, including the writer's, for he had meanwhile completed a book on the subject for publication at the time of transmission. When the film was nearing completion, the words of the final commentary had to be fitted to the pictures at a rate of two short words per foot of film, with allowances for music and effects. This tricky form of writing, somewhat akin to composing telegrams, is a sovereign remedy for prolixity.

About 80% of the programme is on film. The interspersed studio sequences supply a friendly face and a guide to the events of the evening; David Attenborough is the BBC's presenter for *The Life Game*. The studio also serves to explain certain principles or awkward details, to display visual information and to give that sense of drama peculiar to a cleverly designed and lighted electronic studio. Careful thought went into such questions as how the structures of biological macromolecules could best be represented, and how the games idea could be developed in the studio. One invention was a molecular fruit machine (slot machine, in United States usage) to illustrate the difficulty of assembling the first genes unless some kind of 'hold' button was available in the primaevial soup. For the grand gesture in the studio, a large tree diagram, showing evolutionary relationships, was to be painted on the studio floor. On it would stand representations of selected fossil plants and animals and also living animals including—for the Cecil B. De Mille touch—an elephant.

Given this framework of the studio plan, the final studio script could be written and then the work on the scientific content was virtually complete. But in practice the studio, as always, represented the most strenuous phase of the making of the programme, with the creative and technical talents of many people having to take effect simultaneously. We had begun our thinking about the programme by wondering what was wrong with Jacques Monod's philosophy of molecular evolution in *Chance and Necessity*; we finished in a frenzy of anxiety lest the elephant should urinate on the studio floor and destroy the water-based paint of the evolutionary tree.

And what is the 'story' to which I have been referring? Many of its components have been mentioned but I must ask the reader's forgiveness—it takes 150 min to tell.

Hypothesis of Post-recombination Resynthesis of Gene Copies

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Rectification of gene copies by replication of a master copy cannot occur at every meiosis, or substantial DNA synthesis would result. If replication is the method of rectification, the process is likely to be restricted, in any one meiotic cell, to the genes that have undergone recombination.

CALLAN¹ suggested that the gene is accompanied in tandem array by a series of copies of it, and that these "slaves" can

be made identical with the "master" copy—a process now often called rectification—by matching each slave in turn with the master. An alternative to matching against a master as a means of rectifying gene copies is replication from the master as template. Brown *et al.*² discussed this possibility, and suggested that DNA replication by a rolling-circle mechanism³ may be responsible. Buongiorno-Nardelli *et al.*⁴ favoured a similar hypothesis, with the replication restricted to meiosis. They suggested, furthermore, that there may be a regulatory mechanism which determines the number of copies of all the genes showing multiplicity⁵.

Study of the nucleolar organizers—the genes for the major (18S and 28S) RNA component of the ribosomes—in *Drosophila melanogaster* has provided some support for rectification by replication. It seems that the number of copies of the nucleolar

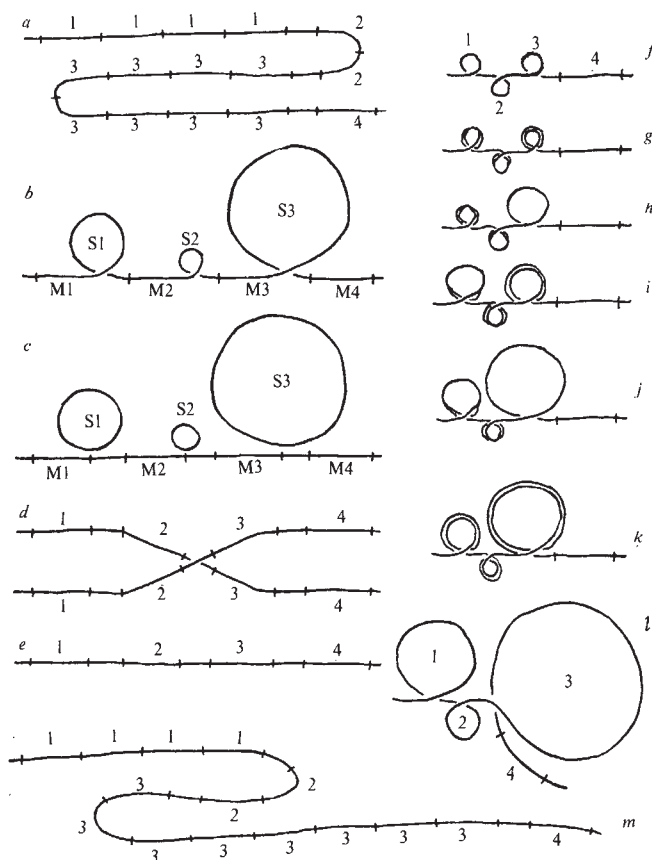


Fig. 1 Diagram to illustrate the hypothesis of rectification of copies of a gene by replication from a master copy after recombination has occurred. The lines represent chromatids at the pachytene stage of meiosis. Four genes are shown and their master copies are labelled M_1 to M_4 and their slaves S_1 to S_4 , respectively. The four genes are represented by a total of four, two, eight and one copies, respectively. *a*, The four genes and their copies as a linear structure. *b*, The same as a cycloid with the slave copies in the loops. *c*, The loops detached by intra-chromatid crossing-over. *d*, A cross-over between the loop-free chromatid segment and a homologous chromatid, likewise without the slave copies of the genes. The oblique segments of the chromatids are intended to indicate the extent of the hybrid DNA segments of the cross-over, shown extending through one gene and into another. *e*, One of the recombinant chromatids. *f-m*, Stages in the formation of copies of genes M_1 to M_3 by successive doubling of the size of a circle of DNA. The rate of replication of each gene is shown as inversely proportional to the number of copies produced, in accordance with the hypothesis of control of numbers by differential rates of synthesis. *g, h*, Genes one to three have completed $2/3$, $1/3$ and the whole of the first doubling, respectively. *i, j*, They have completed $1/3$ of the second doubling, $2/3$ of the first doubling, and the whole of the second doubling, respectively. *k, l*, They have completed two, one and three successive doublings, respectively.

genes in *D. melanogaster* can increase from one generation to the next. The nucleolar organizers in *D. melanogaster* are situated in the X and Y chromosomes and have been shown by hybridization of DNA and rRNA to comprise about 255 and 200 copies respectively of the gene⁶. Mutants deficient in copies of the nucleolar gene have *bobbed* bristles⁷, for example, ninety-eight copies for a severe mutant and 157 for a weak one⁶. Ritossa⁸ and Tartof⁶ found that flies carrying a *bobbed* mutant in their X chromosome and lacking four fifths of their Y chromosome nucleolar organizer give progeny which have only a weak *bobbed* phenotype and have an increased number of copies of the X chromosome nucleolar gene; this effect is progressive so that within three generations the mutants have reverted to wild type. Likewise, Atwood⁹ found that an increase in the number of copies of the Y chromosome nucleolar gene occurs in the progeny of flies which have a *bobbed* mutant in the Y chromosome, and an X chromosome deficient for the entire nucleolar organizer. Moreover, this and

other experiments⁹ have shown that the increase in number of gene copies is not the result of unequal crossing over between nucleolar organizers in homologous chromosomes. These increases in number of copies, apparently compensating for an overall deficiency, can be accounted for if synthesis of a new set of copies continues for one or more rounds of replication beyond the level reached in the previous generation.

Keyl¹⁰ found that the DNA contents of individual bands in the salivary gland chromosomes of subspecies *thummi* of *Chironomus thummi* are either equal to those of the corresponding bands in subspecies *piger* or are two, four, eight or sixteen times greater. These differences can be accounted for by successive doubling of the number of copies of particular genes in the ancestry of subspecies *thummi* during its evolutionary divergence from *piger*, but the absence of any doubling in *piger* is unexplained. This absence, however, is accounted for if there is some overall control of the amount of replication in the production of copies, and if in the *piger* ancestry a general reduction has occurred in this amount.

Miller and Knowland¹¹ discovered that a mutant of *Xenopus laevis* with a small nucleolus has half the normal number of copies of the nucleolar gene, and furthermore synthesizes rRNA at only 25–40% of the normal rate¹². This double defect is particularly interesting. The halving of the number of copies can be accounted for if replication to produce the copies proceeds by a doubling mechanism, as Buongiorno-Nardelli *et al.*⁴ have suggested, and stops one round of synthesis early. Is it possible that the slow rate of synthesis of rRNA in the mutant occurs also for the synthesis of the copies of the gene? Keyl's data¹⁰ for *Chironomus thummi* show that the number of copies of different genes differs considerably, so if it is true, as Amaldi *et al.*⁵ suggest, that there is overall control of the number of copies of all the genes showing multiplicity, the controlling mechanism cannot act on the number of rounds of replication. The rate of DNA synthesis is a possible controlling factor, with a slower rate the smaller the number of copies. The general control of the number of copies of all the genes may then be exercised through the time available at meiosis for the rectifying replication, while changes in the relative number of copies of individual genes, such as Keyl¹⁰ discovered, could operate through the hypothetical mechanism determining the rate of synthesis of copies.

Rectification and Recombination

In view of this support from several sources for the idea that gene copies arise by replication—probably successive doubling or cascade replication, to account for the exponential series^{4,10}—from a master copy, it seems worthwhile to consider the implications of such a hypothesis from the point of view of recombination. In the form proposed by Buongiorno-Nardelli *et al.*⁴ the hypothesis predicts a big turnover of DNA at meiosis, since the tandem array of copies of each gene is synthesized afresh. But there is no evidence for extensive synthesis at this time. Hotta and Stern¹³ found that about 0.1% of the total DNA of *Lilium* pollen mother cell nuclei was synthesized at pachytene. Yet the *Lilium* species they studied has a high DNA content (27.5 pg per haploid set of chromosomes¹⁴ compared, for example, with 3.0 pg in man¹⁵) implying considerable multiplicity of many of its genes. The Buongiorno model also fails to account for some features of recombination. There is evidence^{16–18} that in the recombination process the bringing together of nucleotide chains from the two parents to form hybrid DNA can extend through one gene and into a neighbouring one. Their model does not explain how this can come about, nor how changes of nucleotide sequence resulting from recombination are transmitted to the copies.

It is well established that recombinant genes, for example wild type alleles arising from crosses of two allelic mutants, function in the immediate progeny. If the gene in question has multiple copies, and if it is these copies that are at least predominantly responsible for gene expression, it follows that the

recombinant genotype must be transmitted to the gene copies immediately after it has arisen. In Callan's hypothesis¹ this is taken care of by postulating that the matching process occurs at the diplotene stage of meiosis at the base of each lampbrush loop. For the replication hypothesis it is necessary, therefore, to postulate that the replication to give a set of copies of the gene takes place immediately after recombination has occurred and involves the copy of the gene which has taken part in recombination. To accommodate the requirements for rectification and recombination the following steps seem necessary during pachytene. (1) All the copies of the gene except one are detached from the chromatid as a result of a Campbell-type¹⁹ cross-over²⁰ (Fig. 1*b, c*). (2) The copy of the gene remaining in the chromosome, that is, the master copy, recombines with its counterpart in a homologous chromatid, the hybrid DNA sometimes extending into one or more neighbouring genes (Fig. 1*d*). (3) The master copy then undergoes replication, presumably by a doubling or cascade process, to give a tandem array of copies alongside itself in the chromatid (Fig. 1*f-m*). Replication *in situ* within the chromatid^{2,10} seems more likely than detachment before replication⁴, because there is no evidence from recombination data for reciprocal exchange of whole genes, such as would be expected with simultaneous detachment of a particular gene from two chromatids in close proximity to one another.

Since detachment from the chromosome of all but one of the copies of a gene seems to be required not only for recombination but also for rectification by replication, and in view of the relatively small amount of DNA synthesis at pachytene¹³, it seems likely that rectification at meiosis, if it occurs by replication, is confined to those genes which have been involved in recombination. This conclusion offers a new explanation for one of the recombination phenomena.

Cross-overs and Cross-unders

Study of recombination in fungi²¹⁻²⁴ and in *Drosophila*²⁵⁻²⁷ has revealed that intragenic recombination can occur in conjunction with a parental configuration of genes on either side. Both cross-overs and these non-cross-over recombination events are regularly associated with the phenomena of post-meiotic segregation and conversion. Indeed the only consistent difference between cross-over and non-cross-over events, apart from the presence or absence of an exchange of homologous segments of chromatids, is in their interference pattern: cross-overs usually show positive interference with one another, but non-cross-over events show no interference with cross-overs²⁷⁻²⁹. In view of this similarity of "non-cross-over recombination events" to cross-overs, it would be convenient to denote them by a similar term, and so I suggest "cross-under". This expression also implies something less obvious than a cross-over, which is appropriate since a cross-under, not being associated with exchange of large chromosome segments, does not cause a chiasma, and even genetically can be detected only by the use of closely-linked mutants.

The frequency at meiosis of cross-unders out of total recombination events (cross-unders and cross-overs) has ranged from about 30% in several genes in *Aspergillus nidulans* (review: ref. 30) to about 80% in the *me-7* gene of *Neurospora crassa*³¹. In no experiments have cross-unders been absent. Their regular occurrence raises the question of their significance, since their effect is merely to exchange a few hundred nucleotides between homologous chromosomes. In the presence of cross-overs this effect must be negligible. I have argued³²⁻³⁴ that cross-unders are part of a mechanism for controlling the frequency and distribution of cross-overs in the chromosome. If the recombination events were initially randomly distributed and ambiguous as to type, an enzyme system moving along each chromosome pair at pachytene might switch some events to the cross-under pathway and others to the cross-over pathway, so that the cross-overs were spaced out.

On the other hand, if rectification is confined to genes that

have undergone recombination, the significance of cross-unders might be in maintaining a higher frequency of rectification than the cross-over frequency would allow. Rectification would be necessary from time to time to remove differences between gene copies arising from mutation. The random distribution of cross-unders along chromosomes might then be a response to selection favouring equal opportunities for rectification for all the genes, while selection might act quite differently on cross-overs and favour localizing them and spacing them out.

Testing the Post-recombination Hypothesis

The hypothesis that rectification occurs at pachytene by replication from the copy of a gene which has been involved in recombination could be tested if it were possible to recognize this rectified DNA, for example, by introducing a density label at pachytene and finding segments of DNA labelled in both nucleotide chains. Labelling with ³H-thymidine at pachytene should lead to silver grains at chiasmata in autoradiograms of first metaphase preparations, if the replication is occurring in regions of crossing over. In practice these experiments obviously present formidable difficulties.

If replication of a gene to give a tandem array of copies could be detected from molecular studies, it would be possible to alter the recombination frequency, for example by changes of temperature, and to investigate whether there was a corresponding change in the frequency of localized replication. Likewise, mutants such as *c(3)G* in *Drosophila*³⁵ which suppress recombination would be expected to show no rectification. If the increase in germ line nucleolar gene number^{6,8,9} observed with *bobbed* mutants in genotypes with low numbers of these genes is caused by post-recombination replication, it would be expected not to occur in the *c(3)G* homozygote.

Measurements of the amount of DNA synthesis at pachytene¹³ in related organisms with different DNA contents per nucleus would be instructive. If much of this synthesis relates to rectification, it should form a constant fraction of the total DNA content. On the other hand, if this synthesis is part of the process of recombination, the absolute amount might be expected to be approximately constant, so species with a low DNA content would have a larger fraction synthesized at pachytene than those with a higher content. If the pachytene DNA replication does relate, at least in part, to rectification, and if autoradiograms could be obtained of DNA synthesized at this time, measurements of the rate of synthesis in relation to the size of the replicated segment would test the hypothesis of differential rates as the means of controlling the number of copies of genes.

If rectification by replication is a regular sequel to recombination in any gene with multiple copies, and if the replication is semi-conservative, genes showing multiplicity will not show postmeiotic segregation. Instead, half the copies of the gene will be of one genotype and half of the other, corresponding to the mutational difference between the two chains of the DNA of the master copy of the gene. In consequence, genetic instability might be evident. Although possible examples of postmeiotic segregation are known in *Drosophila*^{26,27}, the phenomenon is well established only in fungi. The discovery of a fungal gene, mutants of which when crossed with wild type failed to show postmeiotic segregation but gave some unstable progeny, would provide support for the hypothesis. Conversely, the occurrence of postmeiotic segregation suggests either that the gene concerned does not show multiplicity, or that rectification is not by replication.

Note added in proof. Smyth and Stern³⁶ have studied the re-annealing kinetics of the DNA synthesized at pachytene in *Lilium* and have concluded that the bulk of it contains nucleotide sequences repeated 2,400 to 3,000 times in each pollen mother cell. This discovery is in agreement with the present hypothesis, although the uniform degree of repetition which they found

is not expected, since different genes are thought to have different numbers of copies.

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- ¹ Callan, H. G., *J. Cell Sci.*, **2**, 1 (1967).
- ² Brown, D. D., Wensink, P. C., and Jordan, E., *J. molec. Biol.*, **63**, 57 (1972).
- ³ Gilbert, W., and Dressler, D., *Cold Spring Harb. Symp. quant. Biol.*, **33**, 473 (1969).
- ⁴ Buongiorno-Nardelli, M., Amaldi, F., and Lava-Sanchez, P. A., *Nature new Biol.*, **238**, 134 (1972).
- ⁵ Amaldi, F., Lava-Sanchez, P. A., and Buongiorno-Nardelli, M., *Nature*, **242**, 615 (1973).
- ⁶ Tartof, K. D., *Genetics*, **73**, 57 (1973).
- ⁷ Ritossa, F. M., Atwood, K. C., and Spiegelman, S., *Genetics*, **54**, 819 (1966).
- ⁸ Ritossa, F. M., *Proc. natn. Acad. Sci. U.S.A.*, **60**, 509 (1968).
- ⁹ Atwood, K. C., *Genetics*, **61**, suppl., 319 (1969).
- ¹⁰ Keyl, H.-G., *Chromosoma*, **17**, 139 (1965).
- ¹¹ Miller, L., and Knowland, J. S., *J. molec. Biol.*, **53**, 329 (1970).
- ¹² Knowland, J. S., and Miller, L., *J. molec. Biol.*, **53**, 321 (1970).
- ¹³ Hotta, Y., and Stern, H., *J. molec. Biol.*, **55**, 337 (1971).
- ¹⁴ Howell, S. H., and Stern, H., *J. molec. Biol.*, **55**, 357 (1971).
- ¹⁵ Mirsky, A. E., and Ris, H., *J. gen. Physiol.*, **34**, 451 (1951).
- ¹⁶ Calef, E., *Heredity*, **11**, 265 (1957).
- ¹⁷ Putrament, A., *Molec. gen. Genet.*, **100**, 321 (1967).
- ¹⁸ Murray, N. E., *Genet. Res.*, **15**, 109 (1970).
- ¹⁹ Campbell, A. M., *Adv. Genet.*, **11**, 101 (1962).
- ²⁰ Whitehouse, H. L. K., *J. Cell Sci.*, **2**, 9 (1967).
- ²¹ Giles, N. H., *Cold Spring Harb. Symp. quant. Biol.*, **16**, 283 (1952).
- ²² Pritchard, R. H., *Heredity*, **9**, 343 (1955).
- ²³ Mitchell, M. B., *Proc. natn. Acad. Sci. U.S.A.*, **41**, 215 (1955).
- ²⁴ Kitani, Y., Olive, L. S., and El-Ani, A. S., *Am. J. Bot.*, **49**, 697 (1962).
- ²⁵ Ballantyne, G. H., and Chovnick, A., *Genet. Res.*, **17**, 139 (1971).
- ²⁶ Chovnick, A., Ballantyne, G. H., and Holm, D. G., *Genetics*, **69**, 179 (1971).
- ²⁷ Carlson, P. S., *Genet. Res.*, **17**, 53 (1971).
- ²⁸ Stadler, D. R., *Proc. natn. Acad. Sci. U.S.A.*, **45**, 1625 (1959).
- ²⁹ Fogel, S., and Hurst, D. D., *Genetics*, **57**, 455 (1967).
- ³⁰ Whitehouse, H. L. K., and Hastings, P. J., *Genet. Res.*, **6**, 27 (1965).
- ³¹ Murray, N. E., *Genetics*, **61**, 67 (1969).
- ³² Whitehouse, H. L. K., *Biol. Rev.*, **45**, 265 (1970).
- ³³ Whitehouse, H. L. K., *Brookhaven Symp. Biol.*, **23**, 293 (1972).
- ³⁴ Ahmad, A. F., Bond, D. J., and Whitehouse, H. L. K., *Genet. Res.*, **19**, 121 (1972).
- ³⁵ Smith, P. A., and King, R. C., *Genetics*, **60**, 335 (1968).
- ³⁶ Smyth, D. R., and Stern, H., *Nature* (in the press).

Intraplate Earthquakes, Lithospheric Stresses and the Driving Mechanism of Plate Tectonics

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Focal mechanisms of about eighty intraplate earthquakes and other information on *in situ* stress indicate that the interiors of many lithospheric plates are characterised by large horizontal compressive stresses. These stresses seem to be related to the driving mechanism of plate tectonics.

ABOUT 90% of the world's earthquakes occur along the boundaries of lithospheric plates. Focal mechanism solutions of earthquakes have provided a detailed description of the type of faulting and the nature of the displacements for most of the major plate boundaries¹. Another class of shocks, intraplate earthquakes, are less numerous than those along plate boundaries and have received relatively little study. Consequently, their cause and tectonic settings have not been well understood.

Here we attempt to determine the type of faulting and to infer the state of stress within lithospheric plates on a world-wide scale from analyses of focal mechanisms of about eighty earthquakes, most of which are located well away from plate boundaries. Our principal result is that a large percentage of these shocks, particularly those not located near mid-ocean ridges or behind subduction zones, are characterised by a predominance of thrust faulting. In the few areas of the world for which they are available, other determinations of the

state of stress from overcoring, hydrofracturing and recent crustal movements support our conclusion that large areas in the interiors of many lithospheric plates are characterised by high horizontal compressive stresses. These seem to be uniform in orientation over sizable regions for the few cases where sufficiently dense data are available.

The very existence of earthquakes in the interiors of the plates indicates that the approximation that plates are undeformed except at their edges is not completely correct even though this assumption seems to be a good working hypothesis for many purposes. The intraplate earthquakes discussed in this paper are all of shallow focus and are located in areas where lithospheric plates are horizontal. Here we present evidence which indicates that the most consistent parameter in focal solutions of shallow earthquakes remote from plate boundaries is the orientation of one of the principal stresses. Earthquakes beneath deep-sea trenches, which involve normal faulting, and intermediate and deep shocks² are other examples of intraplate events in which the direction of one of the principal stress axes is consistent for various nearby shocks. In contrast, slip vectors, rather than principal stress axes, are consistent for earthquakes along a given plate boundary³.

While intraplate earthquakes are not numerous compared with shocks along active plate margins, intraplate shocks as large as magnitude 7 have occurred in several populated areas including parts of central and eastern North America, Europe, Australia and South Africa. Examples of destructive and large intraplate earthquakes include New Madrid, Missouri 1811-12; Charleston, South Carolina 1886; Cape Ann, Massachusetts 1755; Grand Banks 1929; Meckering, Australia 1968, and several large earthquakes in the St Lawrence Valley⁴.

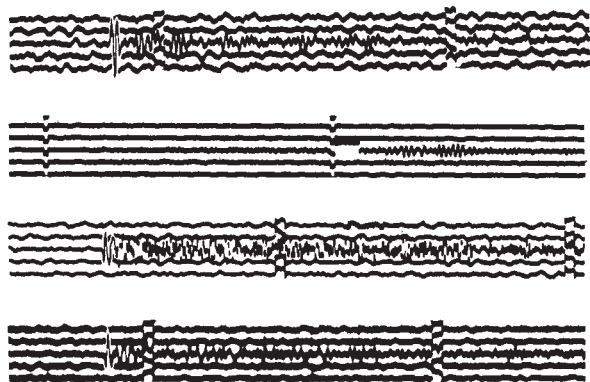


Fig. 1 Short-period vertical seismograms of earthquake near north-east coast of Greenland on November 26, 1971, of magnitude $m_b = 5.2$. Deflexions of trace indicate minute marks, and upward motion of ground is up on records. Station abbreviations, distances and gains are as follows from top to bottom: NUR, 22.8°, 25k; STU, 32.2°, 25k; KBL, 55.7°, 400k; QUE, 59.6°, 200k. The first motion is a clear compression (up) at NUR and a clear dilatational (down) at KBL and QUE. Initiation of P wave and first motion cannot be read at STU, which is inferred to be near a nodal plane. The four stations are labelled on focal mechanism solution in Fig. 2. Arrivals of four P waves are aligned approximately vertically. Light portions of traces are retouched.

These events are characterised by very large areas of perceptibility compared with most shocks of similar magnitudes and energies along plate boundaries. Thus, although they are not numerous, large intraplate shocks constitute an environmental risk that must be taken into account in the design and siting of critical facilities such as nuclear reactors and other large man-made structures. The presence of high compressive stresses in the interiors of many lithospheric plates indicates that the possible triggering of earthquakes by processes that reduce rock strength, such as high-pressure fluid injection and reservoir impounding, must be evaluated carefully.

Large Compressive Stresses

Determinations of stress *in situ*, principally by the overcoring technique, indicate that measured horizontal compressive stresses are much larger than lateral (and even vertical) stresses calculated from the weight of the overburden in Fennoscandia⁵, eastern and central North America⁶⁻⁸, the Colorado Plateau⁹⁻¹⁰ and various parts of the USSR^{11,12}. Thus, the horizontal component of the stress in these areas must be largely tectonic. Although most of these measurements were made primarily for engineering purposes, Voight⁷ recognised that the data may provide important constraints for models of the driving mechanism of large-scale tectonic processes. Nevertheless, very few authors have distinguished between measurements made in what are now recognised as the interiors of plates with those that are pertinent to plate margins.

Sbar and Sykes⁸ examined the directions of principal stresses in central and eastern North America as inferred from *in situ* measurements by the overcoring and hydrofracturing techniques, focal mechanisms, and postglacial geological features. They found good agreement among the various techniques and showed that the greatest principal stress trends nearly ENE throughout a large region that includes much of the eastern and central United States. Focal mechanisms of about ten earthquakes in western Europe north of the Alps are characterised by either thrust or strike-slip faulting such that the axes of maximum compressive stress trend consistently north-west¹³. Thus, in two areas where relatively dense observations of stress are available, the maximum compressive stress seems to be horizontal, to be large, and to have a similar trend throughout a broad region. These factors led us to examine the state of stress within lithospheric plates on a global basis.

Focal Mechanism Data

We examined a magnetic tape file of epicentral locations reported by the National Oceanographic and Atmospheric Administration from 1961 to 1971 for obvious intraplate earthquakes. About half of the seventy-nine mechanism solutions used in this article were determined by us. A list of these events and their mechanism parameters is available from us.

Even though intraplate earthquakes are relatively rare, we were able to obtain a large number of solutions by successfully analysing the first motion of compressional waves for events as small as magnitude $m_b = 5.0$ using standard short-period records. Clear, impulsive first motions of P (Fig. 1) and PKP are often observed on short-period records for intraplate events of $5.0 \leq m_b \leq 5.5$. As many intraplate earthquakes involve dip-slip faulting, stations at distances greater than about 40° (Fig. 2) are often remote from a nodal plane, and therefore record P waves with a distinct first motion. The focal mechanism solution shown in Fig. 2 for an event of $m_b = 5.2$ represents about the average quality of the various solutions reported here.

These factors and the simplicity of the waveform of P indicate that the corner frequency for these events is higher than about 1 Hz and that the instruments "see" a point source rather than the initiation of motion from a complex moving source as is generally the case for $m_b > 5.5$. Most intraplate earthquakes have a larger short-period magnitude, m_b , than do events along plate boundaries of the same long-period magnitude, M_s . The enrichment in short-period energy may be caused by greater effective stresses that may be present within plates and/or higher Q paths through the upper mantle, as intraplate events tend to be located in older (and presumably colder and thicker) lithosphere than events along zones of sea-floor spreading and transform faulting.

Stress Distribution

Figure 3 summarises information on the state of stress as inferred from about eighty focal mechanism solutions of intra-

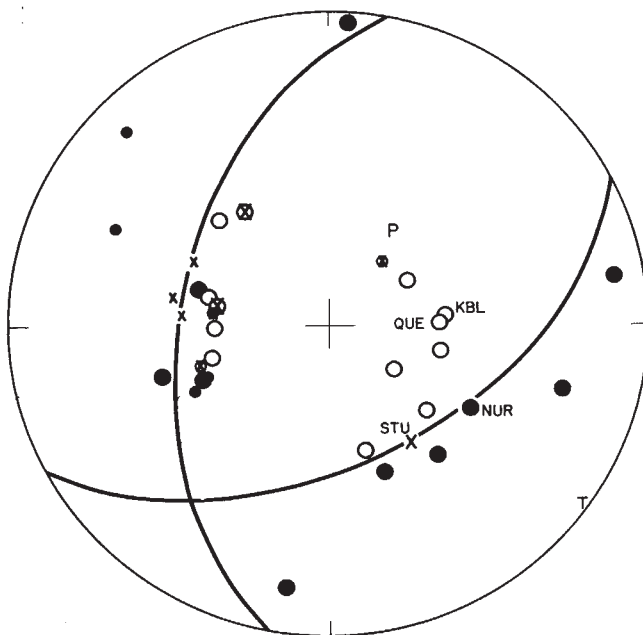


Fig. 2 Focal mechanism (first motion) solution for earthquake near NE coast of Greenland on November 26, 1971. Plot is equal-area projection on lower hemisphere of radiation field. ●, Compressions; ○, dilatations; X, arrivals near nodal plane; P and T are inferred axes of maximum and minimum compressive stress. Large symbols denote more reliable readings. Solution is characterised by a predominance of normal faulting. First motions shown in Fig. 1 are labelled with three-letter codes for stations.

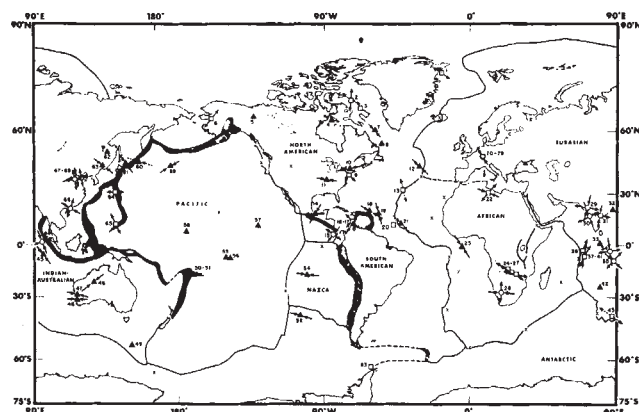


Fig. 3 Worldwide summary of focal mechanism solutions of intraplate earthquakes. $\blacktriangle$, Thrust; $\circ$, strike-slip; $\square$, normal; X, mantle plumes inferred by Morgan^{35,36}. Inwardly directed arrows indicate axis of maximum compressive stress (P axis) and outwardly directed arrows denote least compressive stress (T axis). Dashed arrows indicate less reliable directions. Solution in western Europe is representative of ten mechanisms with similar orientation of P axes. Major plates are labelled. Hatching indicates major subduction zones (Mediterranean-Himalayan zone omitted), and solid line represents plate boundaries of extensional and transform type.

plate earthquakes. The solutions are classified as a predominance of either thrust, strike-slip or normal faulting. The first motions of P and PKP are good enough to support this classification for nearly all the events reported. As in other mechanism studies, we equate the P and T axes of the focal solutions with the axes of maximum and minimum compressive stress. For some of the dip-slip events the azimuths of the nodal planes and of the horizontal principal stress are less reliable. Thus, for these events we show only the type of faulting and not the azimuth of the horizontal principal stress. Although the radiation patterns of surface waves can be used to determine this azimuth with greater precision¹⁴⁻¹⁶, we did not employ it here as substantial additional data analysis is involved.

Table 1 Number of Focal Mechanism Solutions as a Function of Predominant Fault Type

	Thrust	Normal	Strike-slip
Total events (1 to 69, Fig. 3)	34	22	13
Total minus regions behind arcs	31	18	8
Total minus regions behind arcs, close to ridges or in East Africa	29	4	7 (6 in Indian plate)

In the Pacific plate each of the solutions in Fig. 3 is characterised by thrust faulting. In the Atlantic, the solutions for earthquakes located well away from the mid-Atlantic ridge also involve thrusting. Table 1 indicates that thirty-four (49%) of the total solutions in Fig. 3 involve thrusting. The percentage of thrusting increases to 73% when events located near ridges, behind subduction zones or in East Africa are deleted.

Each of the three main types of faulting is found behind active subduction zones, although a single stress pattern usually prevails behind a given arc. For example, focal solutions and other geophysical data indicate horizontal compression in the lithosphere behind the Honshu and South American arcs and extension behind the Bonin-Mariana and Tonga-Kermadec arcs¹⁷⁻²¹. The state of stress behind active subduction zones does not seem to be typical of that in horizontal portions of the lithosphere. Evidence for extension in the Basin and Range province of the western United States is omitted from Fig. 3 as the tectonics of that region does not seem to be intraplate and it may reflect processes associated with a former subduction zone or the cessation of subduction near the west coast of the United States^{22,23}.

Figure 4 shows the type of faulting in oceanic areas as a function of the age of the crust inferred from magnetic anomalies and deep-sea drilling. Thrusting predominates for crust older than 20 m.y.; normal faulting is typical of zones of seafloor spreading at ridge crests and of crust younger than about 10 to 20 m.y. Clearly, the state of stress within the plate has changed from horizontal extension to horizontal compression within the past few tens of millions of years, as it moved away from the ridge crest. The precise age and nature of the boundary between thrusting and normal faulting, however, are not well defined. Six of the eight examples of normal faulting for regions younger than 20 m.y. and not located on ridge crests come from nearly the same location in the Indian Ocean near a large seamount at the southern end of the Chagos-Laccadive ridge²⁴. Although solutions characterised by normal faulting were not found near the East Pacific Rise, the one thrusting solution at 10 m.y. indicates that the transition to normal faulting on that ridge system occurs for crust of younger age.

No examples of normal faulting were found for oceanic crust older than 10 to 20 m.y. Normal faulting was detected for an event in New Jersey and for shocks near the coasts of Greenland, the Antarctic Peninsula, and Baffin Island (Fig. 3). All of these events, however, were located on the continental shelf or within continents and not on oceanic crust. We refrain from further speculation about stresses near continental margins, as the number of events is small.

Although not as clear as in the older oceanic lithosphere, many continental areas, interior to plates, are also characterised by large horizontal compressive stresses. Focal mechanism solutions in Fig. 3 and/or measurements of *in situ* stress corroborate this for western Europe and Fennoscandia^{5,13}, large areas of the USSR^{11,12}, most of the United States east of the Basin and Range Province⁸, western Australia, and peninsular India.

Several examples of normal faulting are found in East Africa (Fig. 3). These events were not located along what are normally regarded as active rifts²⁷. Nevertheless, it is difficult to tell if these solutions are better characterised as intraplate events or as earthquakes associated with a wide and complex plate boundary within a continent. A similar problem was encountered in characterising earthquakes in the broad seismic zone, which probably involves continental collision, in the Mediterranean, Central Asia and the Himalayas²⁶. The mechanism of the Libyan earthquake in Fig. 3 may be related to this broad plate boundary.

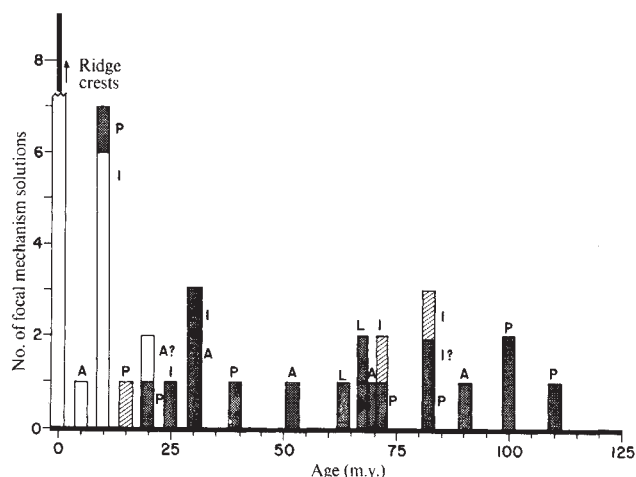


Fig. 4 Type of focal mechanism as a function of age of oceanic crust. Note predominance of normal faulting on, or close to, ridge crests and predominance of thrust faulting for ages greater than 20 m.y. $\square$, Normal; hatched , thrust; diagonal lines , strike-slip type faulting. A, Mid-Atlantic ridge; P, Pacific ridges; L, mid-Labrador sea ridge; I, mid-Indian ridges.

Driving Mechanism

From a compilation of focal mechanism solutions Fitch *et al.*¹⁴ concluded that the orientation of the maximum compressive stress (P axis) varied smoothly throughout the Indian-Australian plate. As the axes of least compression (T axes) for their solutions pointed toward the Java trench, they concluded that gravitational sinking of a lithospheric slab at the subduction zone was an important force driving this plate. All of their solutions in the north-east Indian Ocean, however, involved strike-slip faulting. Additional mechanism solutions for this plate (Fig. 3) indicate thrust as well as strike-slip faulting. The two types of solutions show a similar orientation of the maximum compressive stress for nearby events 44 and 45. An explanation of these data is that the minimum and intermediate principal stresses are of similar size, making possible either thrust or strike-slip faulting. As either one of the minimum and intermediate principal stresses is vertical, they both must be similar in magnitude to the stress resulting from the weight of the overburden. This implies that a significant horizontal tensional component is not being applied to the stress field in this region as a result of gravitational sinking. Instead, the mechanism solutions indicate a large horizontal compressive stress, apparently of tectonic origin.

These conclusions are further supported by thrusting mechanisms (Fig. 3) of shocks located seaward of the Aleutian, Kurile, Tonga and Lesser Antillean arcs. If gravitational sinking were primarily responsible for the stress distribution in plates, normal rather than thrust faulting would be typical of intraplate deformation (Fig. 5a). Earthquakes beneath deep-sea trenches^{1,21,28-30} are typified by normal faulting, but the causative stress seems to be related mainly to the flexure of the lithosphere¹. Normal faulting reported for an event on the outer wall of the Japan trench also seems to be related to flexure rather than to a gravitational sinking mechanism as proposed by Shimazaki³¹. Evidence from topographic and gravity anomalies also indicates great horizontal compressive stresses seaward of several Pacific trenches^{32,33}. The opening of the South Atlantic is also difficult to explain solely by gravitational sinking as the African and Americas plates are not being subducted except in very local areas³⁴. These observations, of course, do not rule out gravitational sinking as a contributory driving force even if it is not the primary driving mechanism. It may well be responsible for the distribution of stress in downgoing plates in island arcs².

Several events in Fig. 3 are located so close to plate boundaries that it is not certain if their mechanisms reflect intraplate stresses or boundary effects. Even though events 50, 51 and 60 are located just seaward of deep-sea trenches, their thrusting mechanisms are distinctly different from normal faulting that

characterises events within trenches. As thrusting is also found elsewhere in the Pacific plate, we interpret these three solutions as intraplate events and not as boundary effects. Events 61, 64 and 65, however, are located behind subduction zones near volcanic arcs. Their mechanisms may be related to the flexure of the lithosphere, other boundary effects, or, in the case of events 64 and 65, to inter-arc spreading²⁰. Event 13 was located about 50 km west of the rift valley and the main seismic zone of the mid-Atlantic ridge²⁵. It is difficult to characterise this event as a plate boundary or an intraplate phenomenon.

Several other mechanisms for driving plates and for stressing their interiors are shown schematically in Fig. 5. Observations of normal faulting close to active ridges and thrusting at greater distances are compatible with plates being driven from below by flow in the asthenosphere or by gravitational sliding or pushing from the general area of ridge crests. The easterly trends of the maximum compressive stress in the south-east Pacific provide evidence for these two mechanisms, as they are nearly perpendicular to the crest of the East Pacific Rise. The trends for solutions 18, 19, 21, and 23 in the Atlantic, however, are nearly parallel to the axis of the mid-Atlantic ridge. Although the trends for these three solutions are not precisely constrained by first-motion data, the uncertainty probably does not exceed 25° for events 19 and 23. Likewise, the maximum compressive stress for events in western Australia, at least one of which is well constrained to be nearly east-west from the radiation pattern of surface waves and from observations of surface faulting¹⁴, is nearly parallel to the axis of the nearby branch of the mid-Indian Ocean ridge. Thus, several solutions do not fit a simple model in which gravitational sliding or pushing from a line source located at the ridge crest is the primary motive force of plate tectonics.

Another possible source of compressive stresses within plates is not related to the driving mechanism, but merely to the gradual cooling and thickening of lithospheric plates (Fig. 5) in that stresses may be frozen in as in the cooling of glass. In oceanic areas the transition from normal to thrust faulting occurs for crust about 10 to 20 m.y. old. This age is nearly the same as the thermal time constant for the formation and destruction of the lithosphere^{1,34}. Nevertheless, we would expect that compressive stresses frozen into the oceanic lithosphere by this mechanism would exhibit some systematic alignment with respect to the direction of lithospheric cooling and thickening. As in the case of gravitational sliding, the P axes of focal mechanisms do not seem to have such a systematic orientation. Near solutions 18, 19 and 23, however, the pattern of sea-floor evolution is not well known and we cannot evaluate this mechanism definitively.

Morgan^{35,36} suggests that mantle plumes are the main driving mechanism of plates. We attempted to test the simple case where each of the plumes he proposes (Fig. 3, X) was of equal strength and the maximum compressive stress was orientated radially about each plume. The orientations of the P axes from focal mechanisms do not obviously correlate with those predicted by the simple model. Also, the directions of principal stresses in Fig. 3 do not seem to correlate with directions of absolute plate motions inferred by Morgan^{35,36} from tracks of hot spots.

Although we cannot isolate a single mechanism as the driving force of plate tectonics, data on the distribution of stress on a worldwide basis provide valuable constraints on proposed mechanisms. We refrain from looking for patterns in the directions of principal stresses in Fig. 3 as the data points are widely spaced except for those in eastern North America and northern Europe where the stress fields do, in fact, appear to be uniform over large areas. Additional focal mechanism solutions and *in situ* stress measurements by the overcoring and hydrofracturing techniques should help to clarify if stress fields generally vary rapidly or are nearly uniform in extent over broad areas in the interiors of most plates. The density of stress determinations in oceanic areas could be greatly

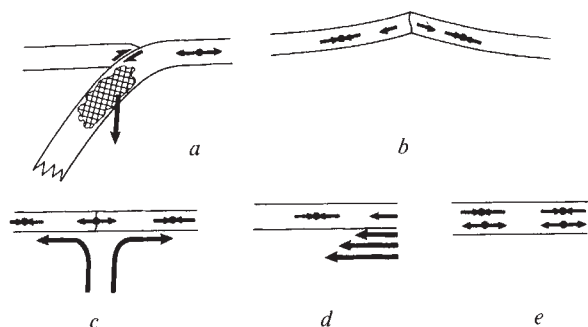


Fig. 5 Schematic diagrams of various mechanisms for generating stresses within lithospheric plates. Single arrows denote relative motions and double arrows denote either compressional (inward) or tensional (outward) deviatoric stresses. *a*, Gravitational sinking in island arc; *b*, gravitational sliding or pushing from ridge; *c*, mantle plume; *d*, rapid flow in asthenosphere; *e*, stresses related to cooling of lithosphere. Hatching in *a* denotes mass excess caused by either cooler material or increased elevation of phase boundaries in sinking slab.

increased by using ocean-bottom seismographs for first-motion studies and by making hydrofracturing measurements in holes drilled into layer 2 of the oceanic crust.

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- ¹ Isacks, B. L., Oliver, J., and Sykes, L. R., *J. geophys. Res.*, **73**, 5855 (1968).
- ² Isacks, B., and Molnar, P., *Rev. geophys. Space Phys.*, **9**, 103 (1971).
- ³ McKenzie, D., and Parker, R. L., *Nature*, **216**, 1276 (1967).
- ⁴ Smith, W. E. T., *Dom. Obs. Pamph.*, **26**, 271 (1962).
- ⁵ Hast, N., *Tectonophysics*, **8**, 169 (1969).
- ⁶ Hooker, V. E., and Johnson, C. F., in *Proc. Fourth Rock Mech. Symp.* (Department of Energy, Mines and Resources, Ottawa, Canada, 1967).
- ⁷ Voight, B., *Am. Assoc. Petrol. Geol. Mem.*, **12**, 955 (1969).
- ⁸ Sbar, M. L., and Sykes, L. R., *Bull. geol. Soc. Am.*, **84**, 1861 (1973).
- ⁹ Raleigh, C. B., Healey, J. H., and Bredehoeft, J. D., *Geophys. Monog.*, **16**, 275 (1972).
- ¹⁰ Haimson, B. C., in *Proc. Fourteenth Symp. Rock Mech.*, 689 (American Society of Civil Engineers, 1973).
- ¹¹ Kropotkin, P. N., *Phys. Earth planet. Int.*, **6**, 214 (1972).

- ¹² Turchaninov, I. A., Markov, G. A., Gzovsky, M. V., Kazikayer, D. M., Frenze, U. K., Batugin, S. A., and Chabdarova, U. I., *Phys. Earth planet. Int.*, **6**, 229 (1972).
- ¹³ Ahorner, L., *Geol. Rdsch.*, **61**, 915 (1972).
- ¹⁴ Fitch, T., Worthington, M. H., and Everingham, I. B., *Earth planet. Sci. Lett.*, **18**, 345 (1973).
- ¹⁵ Mendiguren, J. A., *J. geophys. Res.*, **76**, 3861 (1971).
- ¹⁶ Forsyth, D., *Nature*, **243**, 78 (1973).
- ¹⁷ Ichikawa, M., *Pap. Meteor. Geophys.*, **16**, 104 (1965).
- ¹⁸ Ichikawa, M., *Pap. Meteor. Geophys.*, **16**, 201 (1966).
- ¹⁹ Isacks, B., *Abstr. Prog. Seismol. Soc. Am. Annual Meeting* (1969).
- ²⁰ Karig, D. E., *J. geophys. Res.*, **76**, 2542 (1971).
- ²¹ Katsumata, M., and Sykes, L. R., *J. geophys. Res.*, **74**, 5923 (1969).
- ²² Scholz, C., Barazangi, M., and Sbar, M. L., *Bull. geol. Soc. Am.*, **82**, 2979 (1971).
- ²³ Smith, R., and Sbar, M. L., *Bull. geol. Soc. Am.* (in the press).
- ²⁴ Heezen, B. C., and Tharp, M., *Indian Ocean Floor* (map) (National Geographic Society, Washington DC, 1967).
- ²⁵ Sykes, L. R., *J. geophys. Res.*, **75**, 6598 (1970).
- ²⁶ Molnar, P., Fitch, T. J., and Wu, F. T., *Earth planet. Sci. Lett.* (in the press).
- ²⁷ Maasha, N., and Molnar, P., *J. geophys. Res.*, **77**, 5731 (1972).
- ²⁸ Stauder, W., *J. geophys. Res.*, **73**, 7693 (1968).
- ²⁹ Johnson, T., and Molnar, P., *J. geophys. Res.*, **77**, 5000 (1972).
- ³⁰ Molnar, P., and Sykes, L. R., *Bull. geol. Soc. Am.*, **80**, 1639 (1969).
- ³¹ Shimazaki, K., *Phys. Earth planet. Int.*, **6**, 397 (1972).
- ³² Hanks, T. C., *Geophys. J.*, **23**, 173 (1971).
- ³³ Watts, A. B., and Talwani, M., *Geophys. J.* (in the press).
- ³⁴ McKenzie, D. P., *Geophys. J.*, **18**, 1 (1969).
- ³⁵ Morgan, W. J., *Bull. Am. Assoc. Petrol. Geol.*, **56**, 203 (1972).
- ³⁶ Morgan, W. J., *Geol. Soc. Am., Mem.*, **132**, 7 (1973).

LETTERS TO NATURE

PHYSICAL SCIENCES

Infrared and X-ray Variability of Cyg X-3

CYGNUS X-3 is a candidate for the radio source which in September 1972 experienced a series of exceptional radio outbursts¹. The X-ray emission is chiefly distinguishable as showing periodic intensity variations with a period of 4.8 h (ref. 2); if these variations are caused by an eclipse, the orbital period is the shortest observed up to this time from an X-ray source. The definite identification of the radio source with the X-ray source has remained unconfirmed up to now because of the poor positional accuracy of the X-ray source; the error box in the 3U catalogue is about 1×2 arc min (ref. 3). Although there is no visual candidate for Cyg X-3 to a limit of $\sim 2 \times 10^{-32}$ W m⁻² Hz⁻¹ ($V \sim 23$ mag) (ref. 4), at infrared wavelengths Becklin *et al.*⁵ found a source coincident to ± 2 arc s with the radio position. At 2.2 μ m, the flux density is approximately 2×10^{-28} W m⁻² Hz⁻¹, whereas the 1.65 to 2.2 μ m colour of the infrared source is consistent with a Rayleigh-Jeans spectrum which is reddened by 1.5 mag at 2.2 μ m by the interstellar medium. There is no reason to suppose that the source is intrinsically more luminous in the infrared than at visible wavelengths.

Here we describe the coordinated X-ray and infrared observations at Cyg X-3. These observations have led to the discovery of a synchronous 4.8-h periodicity in the flux density of the infrared source. This uniquely associates the X ray with the infrared source, and hence, through the positional coincidence, with the radio object. In addition, short term variability in the infrared flux of Cyg X-3 is described which may be related to the flares seen at radio wavelengths. An improved estimate of the X-ray position is also given.

The main infrared observations presented here were made

at the Cassegrain focus of the Hale 200-inch telescope on July 9 and 11, 1973, UT. The photometer, using a focal plane chopper to remove background radiation, has been described by Becklin and Neugebauer⁶; both a PbS and an InSb detector were used at 2.2 μ m. The area around Cyg X-3 is crowded, and so a 5 arc s small aperture was used and the photometer oriented to avoid contamination by field stars.

X-ray data were obtained on July 9, 1973, UT, using a collimated proportional counter sensitive in the 1 to 3 Å wavelength range which is part of the MSSL X-ray package on board Copernicus. The system has a field of view of approximately 3° full width at half maximum and an effective area of 17 cm². The counter background is reduced by an anti-coincidence system between the stellar and calibration sections of the detectors, and by pulse-shape discrimination of the output from the stellar channel. The resulting background rate in orbit is of the order of 30 counts per integration period of approximately 62.5 s and this is removed using correlations derived between the 1 to 3 Å guard channel signal and the background level observed when no X-ray emitting object is within the field of view of the detector. The guard channel outputs the number of pulses registered due to the previously mentioned rejection systems. The Copernicus spacecraft provides a stabilised platform which enables continuous observation of a source to be made for extended periods. Breaks in the X-ray data are caused by Earth occultation and passage through the South Atlantic Anomaly.

Following the initial discovery of the infrared object⁵, refined measurements were obtained on the position and size of the infrared source as well as the presence of other infrared sources in the field. The location of the Cyg X-3 infrared candidate was remeasured with respect to visual local field stars which are also infrared sources; four such objects, whose positions were determined with respect to 23 SAO stars, lie within ~ 30 arc s of Cyg X-3. The 1950.0 positions of the infrared and the radio source are given in Table 1.

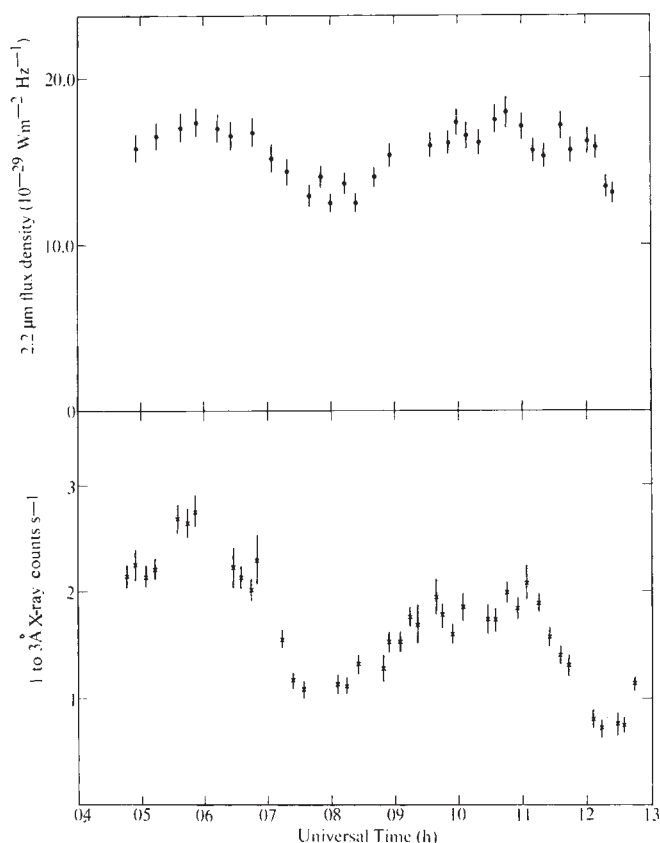


Fig. 1 The 4.8-h periodicity of Cyg X-3. The X-ray data have been averaged over the 6-min integration times of the infrared data. During the time period shown the infrared flux from a comparison star located within 5 arc min of Cyg X-3 was constant within $\pm 2\%$. July 9, 1973.

Measurements of relative fluxes in different sized apertures show the source is less than 3 arc s in diameter. Finally, a map of the area 1.5×1.5 arc min surrounding the source was completed to a flux level of $10^{-28} \text{ W m}^{-2} \text{ Hz}^{-1}$ at $2.2 \mu\text{m}$. No further infrared sources other than the faint visible field stars (see Fig. 1 of ref. 4) were discovered.

Table 1 New X-ray and Infrared Positions (1950.0) of Cyg X-3

	Right ascension			Declination		
X-ray	20 h 30 min	39.5 s	$\pm 3.5 \text{ s}$	$+40^\circ$	47' 00"	$\pm 40''$
Infrared	20 30	37.64	± 0.10	40 47	12.5	± 1.0
Radio	20 30	37.619	± 0.010	40 47	12.62	± 0.15

The radio position is from ref. 7.

Table 1 also gives a new position of the X-ray source as determined with the Copernicus satellite. The error quoted is the radius of uncertainty for 90% confidence. The determination of positions with the Copernicus instrumentation has been described elsewhere⁸.

Figure 1 shows $2.2 \mu\text{m}$ and X-ray observations taken on July 9, 1973, UT, a night on which 9 h of uninterrupted infrared observations were obtained. The periodicity of the X-ray data is duplicated by the infrared data. In particular, both curves are closely in phase although the relative amplitude of the infrared variation is smaller than that of the X rays and the monotonic decrease present in the mean of the X-ray data is apparently missing from the infrared data.

Infrared data taken on July 11, 1973, UT, are shown in two time scales in Fig. 2. The data in the left of Fig 2 present an example of a sharp cutoff from the maximum to the lower flux level. The more detailed, right-hand portion, however, shows that at least two outbursts are seemingly superimposed

on the minimum of the periodic variation; the amplitude of the larger outbursts exceeds the previous maximum almost by a factor of two yet lasts for only slightly more than two minutes. The source was also examined for periodic intensity changes in the range from ~ 0.5 to 150 Hz ; no evidence for such fluctuations was found to a level of $10^{-29} \text{ W m}^{-2} \text{ Hz}^{-1}$.

In addition to the measurements of July 9 and 11, four other, more limited, sets of infrared observations of Cyg X-3 have been made with the 200 inch. Clear evidence for the 4.8 periodicity was seen on five nights between June and August, 1973. Significantly, although the ratio of maximum to minimum flux was the same for all these observations, the mean intensity in June was almost twice that obtained in July. Although on some nights only the 4.8 periodicity is seen, on other nights more unpredictable large amplitude flaring occurs, in one case so intense as to mask any 4.8-h periodic variation.

Measurements of the colour of Cyg X-3 were made at three times during the cycle on July 10, 1973, UT. The data were of low signal to noise ratio because of the redness of the object ($[1.6 \mu\text{m}] - [2.2 \mu\text{m}] \approx 1.0 \text{ mag}$), but there was evidence on the level of two standard deviations that the source became bluer while in its minimum phase.

It is clear that the infrared and X-ray data presented here refer to the same object, and further that the positional agreement of the infrared source with that of the radio source is not accidental. In the rest of this discussion, therefore, we accept the conclusion that the X-ray, the infrared, and the radio emission all originate from the same system.

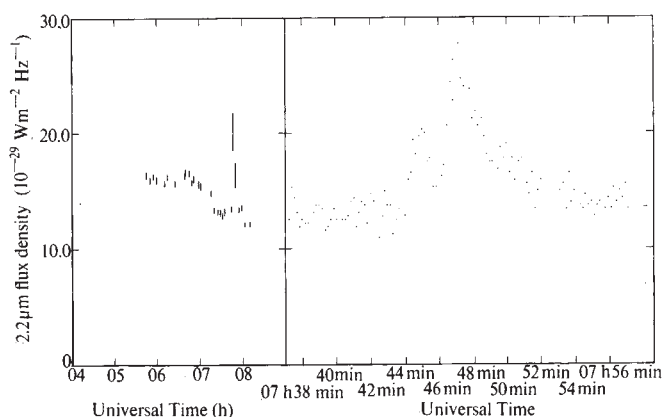


Fig. 2 The infrared flare of July 11, 1973. On the left hand side, the points represent 3-min integrations. A portion of the data at the left is shown broken up into 10-s samples. The statistical accuracy of each point is about 10%.

The fact that the Cyg X-3 displays a similar in phase 4.8-h periodicity at wavelengths ranging from the X-ray to the infrared strongly suggests that it is an eclipsing binary system. Additional evidence in favour of this explanation is provided by: (a) the relative flatness of the infrared maxima; (b) the fact that the relative flux differences between maximum and minimum appear to be constant at different epochs despite changes in both the mean infrared flux and mean X-ray flux; and (c) the fact that the flare activity is apparently uncorrelated with the phase of the 4.8-h variation.

In a binary system with total mass M and period 4.8 h, the separation between the stellar centres is $1.4 (M/M_\odot)^{1/3} R_\odot$. From the shape of the eclipse curve we conclude that the system is a contact, or near-contact, binary with each component of a size not much greater and not much less than one solar radius. By taking the separation of the centres as an upper limit to the diameter of the infrared source, by taking 10 kpc as the lower limit for the distance to the source⁹ and by taking $6 \times 10^{-28} \text{ W m}^{-2} \text{ Hz}^{-1}$ as the dereddened $2.2 \mu\text{m}$

flux density of the source⁵ a lower limit to the 2.2 μm surface brightness temperature is

$$T_B > 6 \times 10^6 (M/M_\odot)^{-2/3} \text{ K}$$

So for any reasonable total mass of the Cyg X-3 binary system, the object seen at 2.2 μm has a surface temperature higher than that of the photosphere of any normal star. Furthermore, given the very high estimated value for the infrared surface brightness, and given the similarity of the X-ray and infrared eclipse curves, it seems extremely probable that both the X-ray and the infrared fluxes originate from the same hot object. For any reasonable value for the total mass of the system, this object has a radius of the order of one solar radius. The observations described in this letter therefore definitely do not favour models for Cyg X-3 involving a compact object such as a white dwarf, a neutron star, or a black hole, and invalidate the suggestion⁵ that Cyg X-3 has anything to do with a BO supergiant.

Perhaps a more plausible prototype for Cyg X-3 is the model discussed by Neugebauer *et al.*¹⁰ to explain Sco X-1. From X-ray, optical and infrared measurements, these authors produced a model for this source consisting of a cloud of thermally radiating plasma with a temperature of $5 \times 10^7 \text{ K}$, optically thick at infrared wavelengths but optically thin at visible and X-ray wavelengths. Cyg X-3 could consist of a somewhat similar, but appreciably larger, cloud of plasma. Cyg X-3 differs from Sco X-1 in being relatively much more powerful at infrared and radio wavelengths in comparison to the X-ray wavelengths; intrinsically, of course, it is also a much brighter object both in the infrared and X-ray wavelengths.

Because Cyg X-3 displays unprecedentedly violent temporal behaviour at both infrared and radio wavelengths it seems very likely that the outbursts seen in the different energy ranges are part of the same phenomenon. It is thus tempting to speculate that the infrared flare is an early manifestation of the synchrotron emission which is often used to explain the radio bursts. Further observations correlating the X-ray, infrared and radio behaviour are clearly needed and are now in progress.

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¹ Gregory, P. C., Kronberg, P. P., Seaquist, E. R., Hughes, V. A., Woodsworth, A., Viner, M. R., and Retallack, D., *Nature phys. Sci.*, **239**, 440 (1972).

² Canizares, C. R., McClintock, J. E., Clark, G. W., Lewin, W. H. G., Schnopper, H. W., and Sprott, G. F., *Nature phys. Sci.*, **241**, 28 (1973).

- ³ Giacconi, R., Murray, S., Gursky, H., Kellogg, E., Schreier, E., Matilsky, T., Koch, D., and Tananbaum, H., *Astrophys. J.* (in the press).
- ⁴ Westphal, J. A., Kristian, J., Huchra, J. P., Shectman, S. A., and Brucato, R. J., *Nature phys. Sci.*, **239**, 134 (1972).
- ⁵ Becklin, E. E., Kristian, J., Neugebauer, G., and Wynn-Williams, C. G., *Nature phys. Sci.*, **239**, 130 (1972).
- ⁶ Becklin, E. E., and Neugebauer, G., *Astrophys. J.*, **151**, 145 (1968).
- ⁷ Branson, N. J. B. A., Martin, A. H. M., Pooley, G. G., Readhead, A. C. S., Shakeshaft, J. R., Slingo, A., and Warner, P. J., *Nature phys. Sci.*, **239**, 133 (1972).
- ⁸ Hawkins, F. J., Mason, K. O., and Sanford, P. W., *Nature phys. Sci.*, **241**, 109 (1973).
- ⁹ Braes, L. L. E., Miley, G. K., Shane, W. W., Baars, J. W. M., and Goss, W. M., *Nature phys. Sci.*, **242**, 66 (1973).
- ¹⁰ Neugebauer, G., Oke, J. B., Becklin, E. E., and Garmire, G., *Astrophys. J.*, **155**, 1 (1969).

Curium-248 in the Early Solar System

PRIMITIVE meteorites such as carbonaceous chondrites (CC) and unequilibrated ordinary chondrites (UOC) have an anomalous fission xenon component¹⁻³ which has a distinctly different fission spectrum from that of Ca-rich achondrites^{4,5}. Although the latter is established as due to ²⁴⁴Pu spontaneous fission⁴, the origin of the former component is still debated. Further, the amount of fission xenon present in CC II-IV and some UOC is about two orders of magnitude^{6,7} higher than that of Ca-rich achondrites and even the maximum value of ²⁴⁴Pu could account only for 10% of CC fission xenon. Several mechanisms, such as carrier hypothesis⁸, mass fractionation⁹, superheavy elements^{6,7}, and two-component trapped xenon¹⁰, have been proposed to explain the CC fission xenon. Here we attempt to determine whether some actinide isotopes with known properties could produce this excess fission xenon in the primitive chondrites.

In Fig. 1, measured ¹³⁰Xe/¹³²Xe values for unmetamorphosed chondrites—Renazzo¹¹, Allende¹², Makoia and Chainpur⁸ and Leoville⁹—are plotted against ¹³⁶Xe/¹³²Xe values after appropriate spallation correction for ¹³²Xe. Here the line joining the primordial point, P, with the point $F=2.4$ for ¹³⁶Xe/¹³²Xe (ratio representing pure fission component) seems to fit most of the experimental points well. Similarly ¹³⁰Xe/¹³²Xe against ¹³⁴Xe/¹³²Xe plots were constructed and

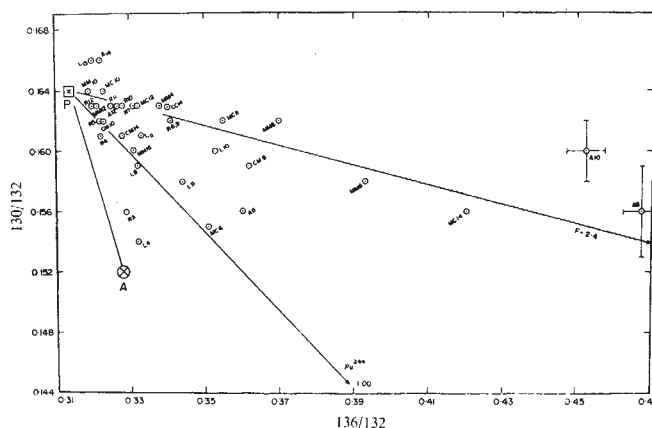


Fig. 1 Correlation diagram between ¹³⁶/132 and ¹³⁰/132 in CC II-IV and UOC for fission component analysis. All the data are corrected for spallation and the points are labelled by release temperatures in hundreds of °C. A, Allende; L, Leoville; CM, Chainpur matrix; CC, Chainpur chondrites and the suffixes are the same for M, Makoia; R, Renazzo. Most of the points (excluding low temperature points where some atmospheric component exists) seem to fit to a fission component given by $(^{136}\text{Xe}/^{132}\text{Xe})_f = 2.4$. For comparison, the line joining P with ²⁴⁴Pu fission component, given by $(^{136}\text{Xe}/^{132}\text{Xe})_f = 1.0$, is also shown. Similar plots were constructed for ¹³⁰/132 against ¹³⁴/132.

there the experimental points fit a line joining P with $F=1.9$ for $^{134}\text{Xe}/^{132}\text{Xe}$. Thus the graphically derived composition of the fission component is 131:132:134:136:0.2:0.41:0.79:1.0. This spectrum agrees well with the values derived by others (Table 1).

Table 1 Fission Xenon Spectra for Carbonaceous Chondrites as Derived by Several Workers and for ^{244}Pu and ^{248}Cm as Obtained by Nuclear Fission Experiments

Fission source	131	132	134	136	Ref.
CCF (as derived from meteoritic studies)					
(a) Pepin spectrum	0.20	0.37	0.70	1.0	1
(b) Marti spectrum	—	0.28	0.71	1.0	2
(c) Geiss spectrum	0.25 ± 0.15	0.38 ± 0.21	0.64 ± 0.10	1.0	3
(d) This work	0.20	0.41	0.79	1.0	This work
Spectrum as derived from laboratory fission studies					
(e) ^{244}Pu	0.25	0.87	0.92	1.0	4
(f) ^{248}Cm	0.30	0.43	0.77	1.0	This work
(g) ^{244}Cm	0.4	0.55	0.89	1.0	4

A study of all the actinide isotopes reveals that only ^{248}Cm is of interest here. For this nuclide, the α half-life and the spontaneous fission (SF) half-life (0.4 m.y. and 5.0 m.y. respectively) are well known, but the fission spectrum is less well known. So we examine in detail the mass distribution of several Cm and Cf isotopes for evaluating the fission spectrum of ^{248}Cm . In Fig. 2, we give the fission spectra of ^{245}Cm (n,f)¹³, ^{248}Cm (SF)¹⁴, ^{249}Cf (n,f)¹⁵ and ^{252}Cf (SF)¹⁶⁻¹⁸, determined by different methods such as mass spectrometry, radiochemistry, and kinetic energy measurements. In Fig. 2b and 2c, the mass yield data are normalised to the ^{132}Xe value. From general fission systematics, the SF spectrum of ^{248}Cm is expected to be similar to those of its even-even neighbours $A=246$, 250 and 252, and Fig. 2a shows that the ^{252}Cf spectrum is similar to that of ^{248}Cm . Also, the ^{250}Cf SF spectrum shows similar mass yield characteristics¹⁶⁻¹⁸. So the relative distribution of $A=131$, 134 and 136 for ^{252}Cf and ^{248}Cm is expected to be similar, provided no fine structure exists in the mass distributions (as we discuss later). To compare the data of several workers, we use a yield value of 2.6% for ^{132}Xe normalisation. The average values are: for $A=136$, 6.0; for $A=134$, 4.7; and for $A=131$, 1.8. The fission spectrum so derived for ^{248}Cm is 131:132:134:136::0.30:0.43:0.77:1.0, which is in good agreement with the graphically derived fission xenon spectrum of the above meteorites.

A brief comment about the fine structure effects in the fission of Cm and Cf isotopes is in order. Harbour *et al.*¹⁶⁻¹⁸ made a detailed comparison for all the mass chains between $A=131$ and 137, a region in which definite fine structure exists in the mass distributions of ^{235}U (n, f) and ^{244}Pu (SF) and found no fine structure effect in ^{252}Cf fission. Also ^{249}Cf (n, f)¹⁵ reveals that it has all the fission characteristics of lighter actinides, except the fine structure in the mass range $A=131$ to 137. The ^{245}Cm (n, f)¹³ also shows no fine structure in the relevant mass range, and the ^{244}Cm (SF)²⁰ shows that the parameter of rigidity against deformation of fission fragments begins to diminish after ^{244}Cm ; this nuclide seems to be in the transition region for fine structure between the lighter and heavier actinides. So for ^{248}Cm (SF), the mass yield curve rises steeply between masses 131 and 137 (Fig. 2).

The amount of fission gas present in CC II-IV and UOC is about $70 \times 10^{-12} \text{ cm}^3 \text{ }^{136}\text{Xe STP g}^{-1}$ and this is about fifty times greater than that of ordinary chondrites. This large

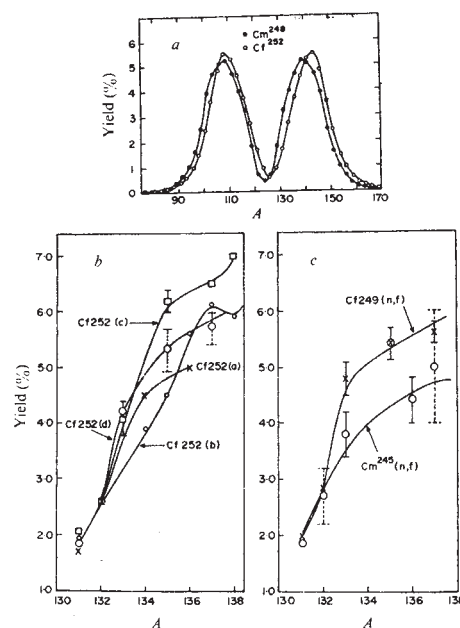


Fig. 2 Experimental fission spectra of several Cm and Cf isotopes as determined by different methods. a, Total mass yield curve for the spontaneous fission of ^{248}Cm and ^{252}Cf , determined by solid state detectors. b, Spontaneous fission mass yield curve for ^{252}Cf determined by, a, Srinivasan *et al.* (mass spectrometry); b, Schmitt *et al.* (solid state detectors); c, Nervik; d, Harbour *et al.* (radiochemistry)¹⁶⁻¹⁸. Thermal neutron fission mass-distributions for ^{245}Cm and ^{249}Cf are given in c. In b and c, all the yields are normalised to a ^{132}Xe value of 2.6%. ^{250}Cf SF spectra (not shown here) are similar to the curves in a.

excess cannot be produced by ^{244}Pu and so we explore the possibility of some other nuclide, such as ^{248}Cm . If we assume that the geochemical behaviour of U, Pu and Cm is similar (as discussed later), it can be written,

$$(^{136}\text{fXe})_{248} = \gamma_1 [^{238}\text{U}] \exp(\lambda_{238}(T + \Xi)) Y_{136}(\lambda_f(\lambda_{248} + \lambda_t))_{248} \exp(-\lambda_{248}\Xi) \quad (1)$$

where γ is $(^{248}\text{Cm}/^{238}\text{U})_0$ at the end of nucleosynthesis, that is this value is taken to be similar to the maximum value²¹ of 1/30 for $(^{244}\text{Pu}, ^{238}\text{U})_0$; λ_{238} the α decay constant of ^{238}U ; T the age of meteorites (4.55×10^9 yr); γ_{136} the cumulative fission yield for 136 mass chain for ^{248}Cm (6%); λ_{248} and λ_{248} the α and spontaneous fission decay constants for ^{248}Cm ($1.732 \times 10^{-6} \text{ yr}^{-1}$ and $0.1386 \times 10^{-6} \text{ yr}^{-1}$, respectively); $[^{238}\text{U}]$ the uranium concentration, an average value of 10 parts per thousand million for chondrites, and Ξ is the time interval between the end of nucleosynthesis and the onset of fission xenon retention in high temperature condensates. If the CC fission xenon ($\sim 7 \times 10^{-11} \text{ cm}^3 \text{ }^{136}\text{Xe STP g}^{-1}$) is produced by ^{248}Cm spontaneous fission, the elapsed interval, Ξ , is about a million years. This time interval is different from the formation interval inferred from I-Xe systematics, as discussed later.

In equation (1), only the value for γ is not known. It is now well established that the heavy elements, Th, U and Pu, are produced in the r-process nucleosynthesis in supernova explosions. Wasserburg *et al.*²² and Hohenberg²³ showed that most of the Th and U isotopes are synthesized in a giant explosion very early in the formation of our Galaxy, $\sim 8 \times 10^9$ yr ago. Also they showed that a ratio of 1/30 for $(^{244}\text{Pu}/^{238}\text{U})_0$ is compatible with a two-spike model of nucleosynthesis where the second spike—possibly a local supernova event which took place almost immediately before the formation of our Solar System—contributed most of the r-process short lived nuclides, ^{244}Pu and ^{129}I . It seems probable that any r-process synthesis which produces heavy elements up to ^{244}Pu should also produce ^{248}Cm —the next even-even element after Pu. Further,

^{248}Cm has a magic neutron configuration $N=152$ and the production of this nuclide consequently will be favoured in r-process nucleosynthesis. So the assumption of $(^{244}\text{Pu}/^{238}\text{U})_0 \approx (^{248}\text{Cm}/^{238}\text{U})_0$ is justifiable.

Additional insight into the problem is provided by the fission of krypton. Low energy asymmetric fission of heavy elements yields both ^{86}Kr and ^{136}Xe with their ratio varying inversely as Z^2/A of the fissioning nuclide²⁴. The measured $^{86}\text{Kr}/^{136}\text{Xe}$ values for different nuclides are the following: ^{235}U (n, f) 0.3, ^{238}U (SF) 0.18, ^{239}Pu (n, f) 0.12, ^{241}Pu (n, f) 0.10, ^{245}Cm (n, f) 0.06 and ^{252}Cf (SF) 0.03 (refs 13, 16–18, 24). It is therefore reasonable to assume this ratio to be very close to 0.10 for ^{244}Pu (SF) and 0.06 for ^{248}Cm (SF). In the Pasamonte achondrite⁵ where the fission xenon is ascribed to ^{244}Pu (SF), the ratio $^{86}\text{Kr}/^{136}\text{Xe}$ is on average about 0.10 in good agreement with the inferred value above. For carbonaceous chondrites, on the other hand, Eugster *et al.*³ have estimated this ratio to be about 0.06 in close agreement with the inferred value for ^{248}Cm (SF), thus lending support to the ^{248}Cm hypothesis. Final validity of this argument has to await more precise fission krypton measurements.

The absence of excess fission gas in ordinary chondrites calls for an explanation. These chondrites reveal recrystallisation to different degrees, probably on account of their metamorphic heating after their primary accretion²⁵. It is conceivable that this heating took place sometime after the complete decay of the 0.4 m.y. ^{248}Cm but before a considerable decay of the 82 m.y. ^{244}Pu so that one finds only the ^{244}Pu fission gas in the ordinary chondrites. The relatively "sharp isochronism" evident from the correlation of radiogenic ^{129}Xe and ^{127}I in the ordinary chondrites presumably refers to the onset of xenon retention in them following the above heating event. The CC and UOC meteorites have escaped completely or partially the above heating event so that they retain the record of curium presence in an earlier stage of Solar System evolution. Since the spontaneous fission decay of ^{248}Cm is about 110 times more dominant than that of ^{244}Pu , the spectrum of the excess heavy xenon in the primitive meteorites resembles essentially ^{248}Cm fission gas spectrum.

It is significant that some CC and UOC meteorites show large excesses of radiogenic xenon, ^{129}Xe ($\sim 1 \times 10^{-9} \text{ cm}^3 \text{ STP g}^{-1}$) and some individual phases yield $^{129}\text{Xe}/^{132}\text{Xe}$ ratios as high as 300 (refs 26–30). But unlike the ordinary chondrites, the radiogenic xenon in these meteorites does not correlate with ^{127}I and the scatter of experimental points is such that they can be fitted to different correlation lines, corresponding to a spread in their apparent ages of about 10 m.y. Further, Podosek and Lewis point out that the formation time of Allende white inclusions is essentially indistinguishable from that of Karoonda and they predate most other chondrites²⁸. The absence of the ordinary chondrite-type correlation in these primitive meteorites has been explained in several ways, such as due to the cold assembly and preservation of phases with individual xenon retention records before the sharply isochronous event in the ordinary chondrites³¹. The I-Xe problem in these primitive chondrites is complicated and no satisfactory explanation exists. But in the light of the arguments presented so far, it appears that the I-Xe and Cm-Xe systematics relate to different events in the early history of our Solar System in that the former refers to the time when I-Xe clocks are reset during metamorphism of the ordinary chondrites whereas the latter refers to the time of primary condensation of the refractory materials from the cooling solar nebula³².

The occurrence of the short-lived nuclide ^{248}Cm ($T_{1/2} = 0.4 \text{ m.y.}$) in the primitive meteorites implied by the foregoing arguments bears importantly on the time scales of the earliest events in the evolution of our Solar System. When the solar nebula is cooling from about 2,000 K to 1,400 K, that is to the temperature where iron and magnesium silicates condense, the high temperature minerals (or phases) form as refractory condensates^{26–28}. Curium, being a refractory element similar

to U, Pu and rare-earths, is most likely to be incorporated into these minerals and they preserve the spontaneous fission record of ^{248}Cm when they cool fast to the xenon retention temperature. Consequently, this actinide isotope fixes the time scale for this fast-cooling stage. In other words, if the time interval for condensation is about a couple of million years, the ^{248}Cm record could be preserved in these early condensates, but if it runs to tens of millions of years, ^{248}Cm will completely decay out before it is incorporated into these minerals. Further, since ^{248}Cm is synthesized only in the r-process and is relatively short-lived, it has to be freshly produced in a "last minute synthesis" similar to ^{129}I and ^{248}Pu and added to a well stirred nebula before the onset of condensation^{22,23}. Some recent theoretical calculations on time scales for condensation of planets and the formation of the Solar System by Cameron³¹ and Hoyle and Wickramasinghe³² support this suggestion. It will be interesting to look for excess fission tracks in primitive chondrites in further support of curium hypothesis.

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- ¹ Pepin, R. O., in *Origin and Distribution of the Elements*, 379 (Pergamon Press, 1968).
- ² Marti, K., *Meteoritics*, **5**, 208 (1970).
- ³ Eugster, O., Eberhardt, P., and Geiss, J., *Earth planet. Sci. Lett.*, **1**, 99 (1966).
- ⁴ Alexander, jun., E. C., Lewis, R. S., Reynolds, J. H., and Michel, M. C., *Science*, **172**, 837 (1971).
- ⁵ Hohenberg, C. M., Munk, M. N., and Reynolds, J. H., *J. geophys. Res.*, **72**, 3139 (1967).
- ⁶ Anders, E., and Heymann, D., *Science*, **N.Y.**, **164**, 821 (1969).
- ⁷ Rao, M. N., *Nucl. phys.*, **A140**, 69 (1970).
- ⁸ Rowe, M. W., *Geochim. cosmochim. Acta*, **32**, 1317 (1968).
- ⁹ Manuel, O. K., Wright, J. R., Miller, D. K., and Kuroda, P. K., *J. geophys. Res.*, **75**, 5693 (1970).
- ¹⁰ Manuel, O. K., Hennecke, E. W., and Sabu, D. D., *Nature phys. Sci.*, **240**, 99 (1972).
- ¹¹ Reynolds, J. H., and Turner, G., *J. geophys. Res.*, **69**, 3226 (1964).
- ¹² Manuel, O. K., Wright, J. R., Miller, D. K., and Kuroda, P. K., *Geochim. cosmochim. Acta*, **36**, 961 (1972).
- ¹³ Harbour, R. M., and MacMurdo, K. W., *J. inorg. nucl. Chem.*, **34**, 2109 (1972).
- ¹⁴ Reinhard, B., Thompson, S. G., Raymond, C. G., and Llad, P., *Phys. Rev.*, **131**, 2617 (1963).
- ¹⁵ Kurchatov, B. V., Movozov, L. N., Pchelin, V. A., and Shubko, V. M., *Soviet J. nucl. Phys.*, **14**, 528 (1972).
- ¹⁶ Harbour, R. M., Eicher, M., and Troutner, D. E., *Radiochim. Acta*, **15**, 146 (1971).
- ¹⁷ Srinivasan, B., Alexander, jun., E. C., Manuel, O. K., and Troutner, D. E., *Phys. Rev.*, **179**, 116 (1969).
- ¹⁸ Nervik, W. E., *Phys. Rev.*, **119**, 1685 (1970).
- ¹⁹ Schmitt, H. W., Kiker, W., and Williams, C. E., *Phys. Rev.*, **137**, 837 (1965).
- ²⁰ Alkhazov, I. D., Kostochkin, O. I., Malkin, L. Z., and Petrzhak, K. A., *Soviet J. nucl. Phys.*, **11**, 281 (1970).
- ²¹ Wasserburg, G. J., Huneke, J. C., and Burnett, D. S., *J. geophys. Res.*, **74**, 4221 (1969).
- ²² Wasserburg, G. J., Schramm, D. N., and Huneke, J. C., *Astrophys. J. Lett.*, **157**, L91 (1969).
- ²³ Hohenberg, C. M., *Science*, **N.Y.**, **166**, 212 (1969).
- ²⁴ Hyde, E. K., *The Nuclear Properties of the Heavy Elements, III, Fission Phenomena* (Prentice Hall, 1964).
- ²⁵ Anders, E., *A. Rev. Astr. Astrophys.*, **9**, 1 (1971).
- ²⁶ Fireman, E. L., DeFelice, J., and Morton, E., *Geochim. cosmochim. Acta*, **34**, 873 (1970).
- ²⁷ Marti, K., *Proc. Thirty-fifth Meteoritical Society Meeting* (Chicago, 1972).
- ²⁸ Podosek, F. A., and Lewis, R. S., *Earth planet. Sci. Lett.*, **15**, 101 (1972).
- ²⁹ Podosek, F. A., and Hohenberg, C. M., *Earth planet. Sci. Lett.*, **8**, 443 (1970).
- ³⁰ Podosek, F. A., *Geochim. cosmochim. Acta*, **34**, 341 (1970).

³¹ Cameron, A. G. W., *On the Origin of the Solar System* (edit. by Reeves, H.) (Nice, 1972).

³² Hoyle, F., and Wickramasinghe, N. C., *Nature*, **217**, 415 (1968).

³³ Herzog, G. F., Anders, E., Alexander, jun., E. C., Davis, P. K., and Lewis, R. S., *Science, N.Y.*, **180**, 489 (1973).

Fuel-Coolant Interactions in Submarine Vulcanism

Most volcanic explosions are caused by pressure from within the Earth's crust but some marine volcanoes are more violent and have a character different from volcanoes in apparently similar circumstances on land. Volcanic eruptions in shallow water have associated "hydroexplosions" some of which belong to a wider class of events, which can also occur industrially, called fuel-coolant interactions (FCI). This has also been pointed out by Colgate and Sigurgeirsson¹. Some authors use the terms "thermal interaction" and "vapour explosion" instead of FCI. Here we illustrate the circumstances under which shallow submarine vulcanism produces hydroexplosions, we indicate what characterises industrial FCI, and using a recent theoretical model we calculate the maximum depth at which hydroexplosions in the form of FCI can occur.

The emergence of the new volcanic island Surtsey provided an opportunity for detailed study of shallow submarine vulcanism. Initially jets of tephra rose to heights of about 150 m every 30 s or so. These black jets had a distinctive shape, "cypressoid" or "cock's-tail", and were accompanied by clouds of steam²⁻⁴. During subsequent months these so-called hydroexplosions occurred whenever the sea had easy access to the vent from which the lava emerged. The explosions occurred at irregular intervals varying from a few seconds to a few minutes. After six months, the cone of tephra became coated with a wave resistant skin of lava, which excluded water permanently from the vent; hydroexplosions then ceased. The character of the explosions was clearly governed by the ease of access of external water into the vent⁵.

The 1957 Capelinhos eruption also involved hydroexplosions⁶. The vent was submerged during the early part of the eruption and contact of the rising magma with the seawater resulted in violent hydroexplosions. Later the eruption provided a clear indication of the importance of water in the explosions. Two vents were simultaneously active—one above water producing mild Strombolian eruptions and one below water producing explosions and cypressoid jets⁵.

It may be that the great eruption at Krakatoa in 1883 was primarily a hydroexplosion⁸. Certainly, however, when activity resumed it consisted of cypressoid explosions until the island Anak Krakatoa was built above water; when this had been destroyed by wave action, the explosions recommenced. This cycle of activity was finally broken after 30 yr when lava overflowed from the crater and coated the cone⁷. Just before that, although the core was 350 foot high and the vent shielded from the sea, the cone was sufficiently permeable for seawater to enter the vent and cypressoid explosions continued to occur intermittently⁹.

The accidental spillage of molten materials or liquefied natural gas (LNG) onto water or wet surfaces is a well known industrial hazard⁹⁻¹⁴. For example, when 25,000 pound of molten steel accidentally fell into an open trough of water, the resulting explosion was heard three miles away¹⁰. It is possible that an FCI was involved in the recent fatal explosion in Scotland when 50 ton of molten steel was being poured into a mould¹⁵.

In experiments supported by the US Bureau of Mines, violent interactions were deliberately produced, and in one case 265 l of LNG was spilled onto water. The resulting explosion occurred ~0.1 s after contact between the LNG and the water.

We emphasise that the essence of an FCI is that two molten materials, one (the fuel) much hotter than the other (the

coolant), suddenly come in contact in such a manner that rapid heat transfer takes place to the cooler material which vaporises and expands explosively; physical rather than chemical processes are involved, and a large amount of thermal energy is transferred from the fuel to the coolant. To effect this rapid transfer within the observed time scale of the explosion (a few tens of milliseconds for metal-water explosions¹⁶) an extremely large contact area between fuel and coolant is needed if normal heat fluxes occur.

Hot molten magma can be brought into contact with water by an underwater volcano. As Judd¹⁷ recognised as long ago as 1888, however, such a condition is not necessarily explosive. Nevertheless rapid mixing may sometimes occur and we believe, as do Colgate and Sigurgeirsson¹, that some hydroexplosions are examples of FCI. In addition, when magma from a sub-aerial volcano runs down to the sea, FCI can take place in the form of small "popping" hydroexplosions producing littoral cones.

Board *et al.*¹⁸ and, independently, Buchanan and Dullforce¹⁹ have proposed a model for FCI in which five stages can be discerned: an initial perturbation which causes a bubble to form in the coolant; the expansion of the bubble and subsequent collapse producing a high velocity jet of coolant which penetrates into the fuel; the breakup of that jet of coolant within the fuel thereby increasing the contact area; the simultaneous heat transfer to the jet; and the vaporisation and the formation of a new high-pressure bubble. The process is then repeated cyclically. Under suitable circumstances the maximum bubble size at each cycle increases with time and the overall heat transfer increases rapidly. An important feature of this model is that the mixing of fuel and coolant occurs on a microsecond time scale, which is necessary if the whole explosion is to take place within milliseconds. Other forms of instability present, such as Rayleigh-Taylor or Kelvin-Helmholtz, grow much more slowly for credible velocities and length scales¹⁹. Buchanan and Dullforce predict that the strength of the interaction is reduced as the external pressure is increased and that there exists a threshold pressure above which the interaction cannot occur.

Submarine vulcanism provides a testing ground for this model. The pressure in the sea increases at the rate of 100 bar km⁻¹ to a maximum of about 500 bar. If pressure can inhibit FCI there must be a depth below which such hydroexplosions do not occur.

D. J. B. (unpublished work) has shown that the threshold pressure, P_{th} , is given by

$$P_{th} = 3\alpha^3 / (1 + \alpha + \alpha^2) P_i \quad (1)$$

where P_i is the pressure at which the new bubble is formed during each cycle and

$$\alpha^3 = (3/16) \beta d_c^2 L_c (\rho_c / \rho_l) \quad (2)$$

where L_c and d_c are constants which determine the jet characteristics; for bubble collapse adjacent to a solid wall L_c and d_c are about 0.5 and 0.25 respectively²⁰; ρ_c is the initial density of the coolant and ρ_l is the saturated liquid density of the coolant at the temperature at which the bubble is formed; β is a numerical factor dependent on the method of vaporisation. For heterogeneous nucleation (normal vaporisation at the saturation temperature) β is unity; for homogeneous nucleation (the method of vaporisation as envisaged by the LNG-water explanations) β is 0.33 for an ambient pressure of 1 bar and decreases as the ambient pressure is increased. An upper limit for P_i can be found as follows. We set the saturated vapour and liquid densities as ρ_v and ρ_l and the vapour pressure as P_v . Since the density cannot change instantaneously from ρ_l to ρ_v we suppose that the expansion occurs adiabatically. Thus, using the normally accepted γ value for water, 4/3, P_i can be found. The implicit equation for P_{th} , equation (1), can be solved by iteration giving $P_{th} = 67.5$ bar (heterogeneous nucleation) or 13 bar (homogeneous nucleation).

In terms of a critical depth, d_{th} corresponding to the threshold pressure, this limiting case corresponds to

$$d_{th} = \begin{cases} \sim 700 \text{ m (heterogeneous nucleation)} \\ \sim 130 \text{ m (homogeneous nucleation)} \end{cases}$$

Earlier authors have obtained other estimates of a maximum depth for hydroexplosions. A limiting value can be obtained by noting that the critical point of water is such that the critical pressure of water (216 bar) corresponds to a depth of about 2 km. Below that depth there is only one water phase and quiet effusions of lava are to be expected^{21,22}.

McBirney²² considered hydroexplosions caused by the expansion of water already present in the magma. He found that the maximum depth at which explosive eruptions can occur was a function of the original water content of the magma and over 2% water content would be required to form ash at a depth of 2 km. On the basis of Friedman's²³ figures for laboratory and field measurements of original water content (~0.1%) he concluded that if the magma was originally at 1,000°C, a realistic temperature²⁴, then explosive eruptions would not occur at depths greater than 500 m. McBirney's results depend on an explicit assumption of his—that seawater surrounding the lava will be vaporised but will not cause significant vesiculation within the lava. Buchanan and Dullforce's model for FCI involving cyclic bubble growth and water injection due to high velocity jetting provides a mechanism which is capable of locally raising the water content of the magma to high levels, and does not depend on the solubility of the magma.

Thus submarine hydroexplosions fall into at least two classes: (i) "McBirney" hydroexplosions, which can occur down to some limiting depth d_{McB} which is a function of the original water content of the magma; (ii) FCI, which can occur down to the threshold depth d_{th} , below which the ambient pressure inhibits the interaction.

It is interesting that the criterion used to decide in some laboratory experiments (D. J. B. and T. A. Dullforce, unpublished) whether the FCI explosion was violent or not is the fraction of metal finely divided. If further development of the theory enables the size of the fragments produced to be correctly predicted, then the size of the glassy granules found by Tazieff²⁵ in Ethiopia may enable the violence of the explosion that produced them to be estimated.

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- ¹ Colgate, S. A., and Sigurgeirsson, T., *Nature*, **224**, 552 (1973).
- ² Thorarinnsson, S., *Surtsey* (Almenna Bókafélagid Reykjavik, 1964).
- ³ MacDonald, G. A., *Volcanoes* (Prentice-Hall, New Jersey, 1972).
- ⁴ *Natn. Geog. Mag.*, **143**, 3 (1973).
- ⁵ Tazieff, H. K., *Soc. Belg. Geol. Bull.*, **67**, 13 (1958).
- ⁶ Cook, M. A., *The Science of High Explosives*, 6 (Reinhold, New York, 1958).
- ⁷ Zen, M. T., and Hadikusumo, D., *Bull. Volcanol.*, **27**, 259 (1964).
- ⁸ Decker, R. W., and Hadikusumo, D., *J. geophys. Res.*, **66**, 3497 (1961).
- ⁹ Sallack, J. A., *Pulp and Paper Magazine of Canada*, **56**, 114 (1965).
- ¹⁰ Long, G., *Metal Prog.*, **71**, 107 (1957).
- ¹¹ Lipsett, S. G., *Fire Technol.*, **2**, 118 (1966).
- ¹² Witte, L. C., Cox, J. E., and Bouvier, J. B., *J. Metals*, **22**, 39 (1970).
- ¹³ Enger, T., Hartman, D. E., and Seymour, E. V., *Proc. Cryogenic Eng. Conference* (National Bureau of Standards, Boulder, Colorado, 1972).
- ¹⁴ Yang, K., *Nature*, **243**, 221 (1973).
- ¹⁵ *The Guardian*, March 31 (1973).
- ¹⁶ Buchanan, D. J., and Dullforce, T. A., *Nature*, **245**, 32 (1973).
- ¹⁷ Judd, J. W., in *The Eruption of Krakatoa and Subsequent Phenomena—Report of the Krakatoa Committee of the Royal Society* (edit. by Symons, G. J.), 21 (1888).

- ¹⁸ Board, S. J., Farmer, C. L., and Poole, D. H., *Central Electricity Generating Board Report*, RD/B/2423 (1972).
- ¹⁹ Chandrasekhar, S., *Hydrodynamic and Hydromagnetic Stability* (Oxford University Press, London, 1961).
- ²⁰ Plesset, M. S., and Chapman, R. B., *J. Fluid Mech.*, **47**, 283 (1971).
- ²¹ Ritmann, A., *Vulcani, attività e genesi* (Naples, 1944).
- ²² McBirney, A. R., *Bull. Volcanol.*, **26**, 455 (1963).
- ²³ Friedman, I., *Proc. Int. Symp. Volcanol.*, 12 (Japan, 1962).
- ²⁴ Hermance, J. F., and Grillet, L. R., *Phys. Earth planet Int.* (in the press).
- ²⁵ Tazieff, H., *Science*, N. Y., **168**, 1087 (1970).

Racemization of Amino Acids in Bones

It has been shown that the extent of racemization of amino acids in a fossil can be used with certain limitations to estimate the age of the specimen¹⁻⁶. For this dating work it is necessary to understand the kinetics and mechanism of the reactions involved. Here we show, by kinetic studies using modern bovine bone fragments heated in sealed ampoules for various time periods at several elevated temperatures, that the order of racemization rates of amino acids in bone is aspartic acid>alanine=glutamic acid>isoleucine≡leucine. Fossil bones not contaminated with modern amino acids are shown to have the same sequence of racemization rates.

The kinetic samples and the fossil bones were first carefully cleaned by ultrasonication in dilute hydrochloric acid (~0.2 M); to remove tar, the La Brea bone was refluxed in a benzene-methanol mixture and in benzene before the HCl cleaning treatment. After cleaning, the bones were hydrolysed for 24 h in 6 M HCl and then desalted as described elsewhere^{5,6}. The average ratios of the D and L-enantiomers of the various amino acids were determined by gas chromatography of the N-trifluoroacetyl-(+)-2-butyl esters⁷ on three different capillary columns (150 foot×0.02 inch) coated with (a) Dexsil 400p; (b) UCON-75-H-90,000; and (c) XE-60 or OV 225. The ratios of alloseucine (alleu) to isoleucine (iso) were determined on a Beckman-Spinco Model 118 amino-acid analyser.

The results of the elevated temperature studies are shown in Table 1. The order of the racemization rates in bone is aspartic acid>alanine=glutamic acid>isoleucine≡leucine, which is in agreement with the results obtained for free amino acids in aqueous solution⁸. The first order rate constant (k) for interconversion of the D and L-enantiomers was calculated for the various amino acids from the equation (see refs 4 to 6 for derivation and use)

$$\ln[(1 + (D/L))/(1 - K'_{eq}(D/L))] - \ln[(1 + (D/L))/(1 - K'_{eq}(D/L))]_{t=0} = (1 + K'_{eq}) kt$$

where $K'_{eq}=1$ for amino acids with only one asymmetric centre but is different for amino acids with more than one centre of asymmetry (for isoleucine $K'_{eq}=0.725$; ref. 5).

Figure 1 shows an Arrhenius plot of the calculated values (aspartic acid values are not shown because the high degree of racemization of aspartic acid in the samples made it impossible to calculate accurate rate constants). As can be seen in Fig. 1, the Arrhenius activation energies (E_a) are identical for the racemization reactions of the various amino acids; this is also the case for the reactions in aqueous solution⁹. If the racemization reactions of any two amino acids had been found to have significantly different Arrhenius activation energies, then the extent of racemization of the two amino acids and the kinetic expressions for the reactions could have been used to calculate both the age of the bone and the average temperature to which the bone had been exposed since deposition.

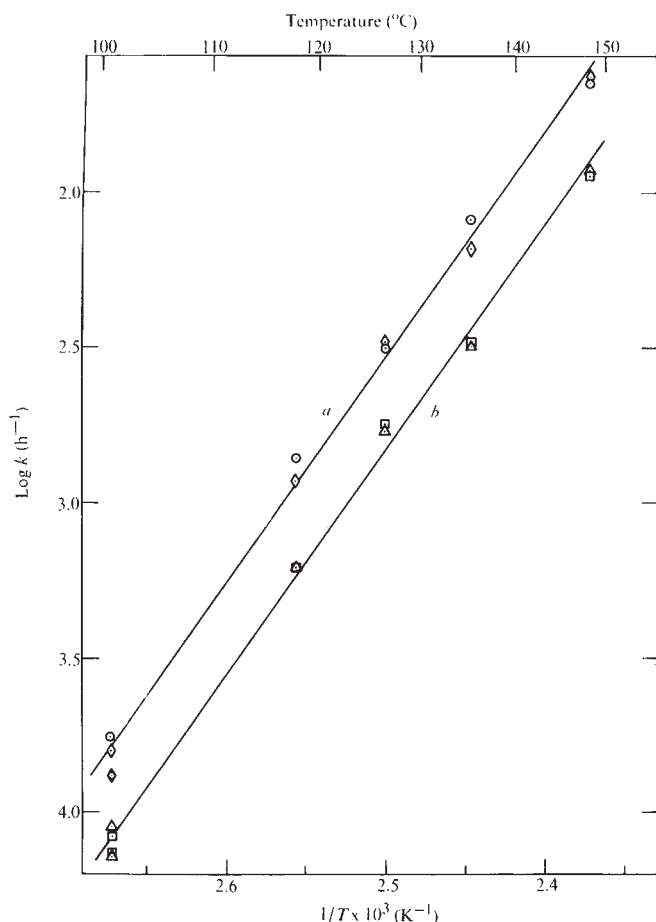


Fig. 1 Log k (h^{-1}) against $1/T$ (K^{-1}) for amino acids in bone: The rate of racemization of an amino acid equals $2k$. The Arrhenius activation energy ($E_a = \text{slope} \times 2.303 R$) for the amino-acid racemization reactions is $33.4 \text{ kcal mol}^{-1}$. $\square$, Leucine; $\triangle$, isoleucine; $\diamond$, alanine; $\circ$, glutamic acid. a , Glutamic acid and alanine; b , isoleucine and leucine.

Unfortunately, this is not the case. To use the extent of racemization of amino acids to estimate the age of fossil bones the temperature history must be evaluated using other information (for example see refs 6 and 10).

The results obtained at 135°C give $k_{\text{iso}}:k_{\text{ala}}:k_{\text{asp}} = 1:2:\sim 7-8$, which agrees with the relative racemization rates (that is, $1:2.4:8.6$) observed in aqueous solution at this same temperature⁸. The amino acids in bones are contained mainly in the fibrous protein collagen. Because the relative rates of racemization of amino acids in bones so closely match those determined for free amino acids in aqueous solution, it would seem that the same factor must influence the rates of racemization of both free and collagen-bound

amino acids. For free amino acids buffered at $\text{pH } 7.6$ the electron-withdrawing capacity of the R-substituents has been suggested as the factor which determines the relative rates of racemization⁸. Evidently, this same factor also determines the relative rates of racemization of amino acids contained in bone collagen.

Since the racemization reactions of amino acids in bone have the same Arrhenius activation energies, the relative order of rates of racemization determined at the elevated temperatures should also exist at the lower temperatures at which fossil bones are found in nature, provided the bone has not been contaminated with amino acids from more recent sources. It is likely that contamination would introduce only L-amino acids to the bone and would thus possibly alter the predicted sequence.

The ratios of D to L-amino acids determined for fossil bones of various ages are summarized in Table 2. These results show that for the bones with ages less than $\sim 60,000$ yr the degree of racemization of the amino acids follows the predicted sequence, but for bones of much older age there are deviations from the expected pattern. Except for the Florisbad bone, the first five samples in Table 2 apparently have not been contaminated with modern amino acids. The pattern in the Florisbad bone is slightly different (that is, alanine > glutamic acid and isoleucine > leucine) from the expected sequence. This deviation may have been caused by the environment (a warm mineral spring)¹⁰ in which this bone was found rather than the result of contamination by modern amino acids.

The Swartkrans sample has an amino-acid racemization pattern suggesting substantial contamination, whereas the Olduvai Gorge sample has a pattern indicating only slight contamination, with only the degree of racemization of aspartic acid seeming too low.

The differences in the degree of preservation of amino acids in fossil bones of various ages can be accounted for by the following mechanism. The collagen content of bones decreases with time; for example, the La Brea bone contains only about a quarter of the amount of organic nitrogen found in modern bones (R. Protsch, private communication) indicating that hydrolysis of collagen and subsequent loss of the amino acids has taken place. Quantitative estimates of the amounts of free, peptide and collagen-bound amino acids in the La Brea bone indicate that essentially all the amino acids are present in the HCl-insoluble, collagen fraction. It seems that free amino acids and small peptides produced from collagen hydrolysis can diffuse out of the bone, and this accounts for the loss of organic nitrogen with time observed in fossil bones.

Amino acids can also diffuse into the bone matrix from the surrounding environment, thus introducing amino acid contaminants. This diffusion process involving amino acids is comparable to the introduction of secondary carbonate into bones and the difficulties it presents in radiocarbon analyses of the total acid-leached carbon in fossil bones¹¹.

Table 1 Racemization of Amino Acids in Modern Bone Fragments Heated at Elevated Temperatures

Temperature ($^\circ \text{C}$)	Heating time (h)	D/L-Aspartic acid	D/L-Alanine	D/L-Glutamic acid	D/L-Leucine	Allee/Iso *
101.1	1,943	0.84	0.30	0.33	0.16	0.19
101.1	2,869	0.91	0.36	0.35	0.21	0.23
117.9	262.5	0.80	0.30	0.35	0.16	0.18
126.4	168.0	0.90	0.50	0.48	0.30	0.31
135.4	48.4	0.83	0.31	0.38	0.16	0.17
148.4	25.1	0.86	0.53	0.51	0.28	0.32
Unheated sample	0	0.15 (0.15)†	0.05	0.07	0.05	0.02

The samples used in this study were taken from the same set used in the investigations of the racemization rate of isoleucine in bone⁵.

* Taken from ref. 5.

† Determined on amino-acid analyser using diastereoisomeric dipeptide method of Manning and Moore¹² modified by Bada and Protsch⁶ for fossil bone analysis.

Table 2 D/L Ratios of Amino Acids in Fossil Bones of Various Ages

Sample location *	Collagen-based radiocarbon age (yr) †	Asp ‡	Ala	Glu	Leu	Alleu/Iso §
Asiab, Iran	8,900 ± 100 (UCLA 1714B)	0.26	0.067	0.080	0.035	0.02
La Brea Tar Pits, California	25,100 ± 1,100 (UCLA 1738F)	0.14	0.055	0.054	0.036	—
Muleta Cave, Mallorca, Spain	28,600 ± 600 (UCLA 1704A)	0.48 (0.46)	0.22	0.26	0.088	0.083
Florisbad Warm Springs, South Africa	38,700 ± 2,000 (UCLA 1745B)	0.82 (0.82)	0.72	0.50	0.30	0.44
Border Cave, South Africa	> 45,000 ¶ (UCLA 1754E)	0.48 (0.46)	0.31	0.26	0.11	0.1
Olduvai Gorge, Tanzania (near top of bed IV)	200,000 to 300,000	0.58 (0.52)	0.48	0.51	0.26	0.30
Swartkrans, South Africa	2 million	0.54	0.37	0.30	0.23	0.37

The degree of racemization of the amino acids is different in bones of about the same age because of differences in average temperatures of the regions where the bones were found.

* With the exception of the La Brea sample, the fossil bones were provided by R. Protsch and R. Berger, University of California, Los Angeles; the La Brea bone was obtained from G. J. Miller, Los Angeles County Museum of Natural History.

† Notation in parenthesis is University of California, Los Angeles Radiocarbon Laboratory identification number.

‡ Ratio in parenthesis is value determined using dipeptide method of Manning and Moore¹² modified by Bada and Protsch⁶ for fossil bone analysis.

§ Ratios determined on Beckman-Spinco amino-acid analyser.

¶ Age estimated to be ≈ 60,000 yr (P. Beaumont, private communication).

For fossil bones such as the Muleta Cave sample, the amount of secondary amino acids is small compared with the amino acids still contained in the indigenous collagen. In very old samples such as Swartkrans, however, the amount of secondary amino acids is significant compared with the small amounts of collagen-bound amino acids remaining in the bone. Indeed, based on the present average yearly temperature of the Swartkrans site (17° C), an age of 2 m.y. would mean that the amino acids should be completely racemized. The substantially lower D to L ratios of the various amino acids in the Swartkrans bone suggests that more than half of the amino acids are of relatively recent origin.

The types of analyses presented here provide a valuable index of the reliability of ages derived from measurements of the degree of racemization of amino acids. For bones like the Muleta or Border Cave samples, contamination by modern amino acids has been shown to be negligible and thus the extent of racemization of amino acids can be used to give reliable age estimates provided the temperature histories of these samples can be evaluated. For a fossil bone such as the Olduvai Gorge sample, which is apparently slightly contaminated with secondary amino acids, the amino acid racemization reaction can still be used to calculate a minimum age; however, for a sample such as the Swartkrans bone, which is badly contaminated, the ages deduced from the degree of amino acid racemization have little significance. By isolating the pure collagen component from the Swartkrans bone the contamination problem could possibly be reduced.

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¹ Hare, P. E., and Mitterer, R. M., *Carnegie Inst. Wash. Yb.*, **67**, 205 (1969).

² Bada, J. L., Luyendyk, B. P., and Maynard, J. B., *Science, N.Y.*, **173**, 907 (1970).

³ Wehmiller, J. F., and Hare, P. E., *Science, N.Y.*, **173**, 907 (1971).

⁴ Bada, J. L., and Schroeder, R. A., *Earth planet. Sci. Lett.*, **15**, 1 (1972).

⁵ Bada, J. L., *Earth planet. Sci. Lett.*, **15**, 223 (1972).

⁶ Bada, J. L., and Protsch, R., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1331 (1973).

⁷ Kvenvolden, K. A., Peterson, E., and Pollack, G. E., in *Advances in Organic Geochemistry* (edit. by Gaertner, H. V., and Wehner, H.), 387 (Pergamon, 1972).

⁸ Bada, J. L., *J. Am. chem. Soc.*, **95**, 137 (1972).

⁹ Bada, J. L., *Adv. Chem. Series*, No. 106, 309 (1971).

¹⁰ Bada, J. L., Protsch, R., and Schroeder, R. A., *Nature*, **241**, 394 (1973).

¹¹ Haynes, V., *Science, N.Y.*, **161**, 687 (1968).

¹² Manning, J. M., and Moore, S., *J. biol. Chem.*, **243**, 5591 (1968).

Measurements *in situ* of Nitric Oxide in the Stratosphere between 17.4 and 22.9 km

A BALLOON-BORNE, nitric oxide sampler was flown on March 16, 1973, from Holloman Air Force Base, New Mexico, 32° 50.1' N, 106° 0.7' W. Measurements were made between 1040 and 1505 local time (1740–2205 UT) over an altitude range between 17.4 and 22.9 km.

The details of the instrument design are given in ref. 1. The principle of the method is based on the chemiluminescence produced when excess ozone is added to a flow of gas containing nitric oxide. The intensity of the radiation detected by the photomultiplier viewing the reaction chamber is given by the expression

$$I = AFX_{\text{NO}}$$

where X_{NO} is the mixing ratio of NO in the sampled air, F is the volume flow rate of the air through the apparatus and A is a constant involving geometry factors of the instrument and rate constants for the chemical reactions involved in the interaction between NO and O_3 . The detection limit for the instrument was 0.02 parts per 10^9 by volume. Provision was made for on-board calibration by addition of known flow of very dilute NO in N_2 . The instrument was turned on and off by ground command. Once on, it was controlled by an internal timer circuit which provided a programme of background signal and then alternate measurements of the ambient air and air to which, successively, one of four flows of calibrating gas was added. Each of these measurements was of duration 2 min. At the completion of the cycle the programme was automatically repeated unless otherwise instructed by ground command.

The photomultiplier output signal was converted into both a linear and a five-decade, logarithmic, photon count rate. A commutated signal was used to monitor other parameters required for housekeeping purposes and for final data reduction. The primary data record was provided by an on-board digital tape recorder. In addition, all signals were relayed to the ground by an S-band telemetry link. Air was drawn into the apparatus through a 2.4 m length of black anodized, aluminium pipe (internal diameter 7.6 cm) and exhausted through a 2.4 m length of flexible tubing (internal diameter 10.2 cm) attached upwards to the payload support lines. The inlet and outlet openings both faced horizontally in opposite directions to prevent changes in air flow resulting from vertical motions of the balloon. The vertical separation of the openings was 2.4 m and the diagonal distance between them was 4.0 m. Laboratory studies in which 0.2 parts per 10^9 of NO were added at either end of the inlet tube to air flowing at $130 \text{ cm}^3 \text{ atm s}^{-1}$ and 27 torr showed that there was negligible adsorption of NO along the tube.

The flight was controlled from the ground to provide three cycles of ascent, float and descent to test for possible contamination from the balloon and flight packages or from the O_3 and NO/N_2 calibration gas used in the measurement. Some forty measurements of the ambient air, each of duration 2 min, were made during the flight. The instrument was turned on by ground control at 17.4 km, the lowest altitude for which the instrument had been calibrated. The maximum altitude reached by the balloon was 22.9 km.

The total error in the measurements is estimated at 60%. The largest error, ~20 to 30%, was due to the small count rates observed, and the balance due to the uncertainties in concentration of the calibrating gas mixture, the total volume flow rate and in the digital tape recording. Within this 60% accuracy, no differences were observed in the measurements taken during ascent, float and descent modes, indicating that there were no serious contamination problems associated with the sampling of the ambient air either from interference from the exhaust or from outgassing of the balloon or instrument package. Most of the measurements were made between 20.1 and 22.9 km. No differences in NO mixing ratio were observed over the altitude range 17.4 to 22.9 km within the experimental error. The average value of the NO mixing ratio was found to be 0.1 parts per 10^9 by volume $\pm 60\%$.

These results agree with those of a previous flight on December 12, 1972, at the same location, at 23.1 km although the data were limited owing to decomposition of the O_3 supply. Other flights at different altitudes and locations are planned. Full details of these measurements and the interpretation of their significance in terms of the SST pollution problem will be reported elsewhere.

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¹ Ridley, B. A., Welge, K. H., Schiff, H. I., Megill, L. R., and Shaw, A. W., *Proc. Second Conf. Climatic Impact Assessment Program*, 146 (1972).

Pollen Evidence for Late Quaternary Climate Changes on Kerguelen Islands

INCREASING evidence indicates that late glacial and postglacial changes in climate and vegetation were essentially synchronous in the northern and southern hemispheres^{1,2}. Pollen analysis of Quaternary deposits on subantarctic ocean islands should provide critical data, but has been inconclusive. The floras of most of the islands are depauperate and yield few identifiable sporomorphs. Species represented in the pollen rain are often of narrow geographical distribution and broad ecological tolerance, thus interpretation of changes in pollen and spore ratios and cross-correlations between islands are difficult³. These problems can be minimized by sampling in a location with a comparatively large flora, with many species widely distributed in the subantarctic, and with a variety of vegetation types. Kerguelen Islands fulfil these requirements.

This compact archipelago, 6,500 km^2 , is located between 49° and 50° S and $68^\circ 30'$ and $70^\circ 30'$ E. The climate is cool and microthermal, with mean temperatures near sea level ranging from 1° C during the coldest months to 8° C during the warmest. Glaciers cover much of the western part of the main island (Fig. 1).

Two cores about 5 cm in diameter were collected for pollen and radiocarbon analyses along stream cuts in gently sloping terrain on the south shore of Golfe du Morbihan (Fig. 1) in early 1971. Both cores included mainly organic-rich silts. There was no clear correlation between soil type and pollen content. The ^{14}C dates obtained from the cores are shown in Figs 2 and 3. In core 1, the two lowest dates present an anomaly, either due to contamination of the lowest sample or the presence of reworked older sediments in the sample above. The anomaly does not seriously affect the interpretation of data from core 1. The bottom of core 1 rested on sediments with rounded boulders, perhaps of glacial origin. If the lowermost organic layers in core 1 result from colonisation of a recently deglaciated area, extrapolation of the ^{14}C dates (Fig. 2) indicates that deglaciation occurred about 10,000 to 12,000 BP. Buried soils found below the bottom of core 2 were not sampled because of seepage problems. If this site was ever glaciated during the late Quaternary, the ice retreated considerably before 11,000 BP (Fig. 3).

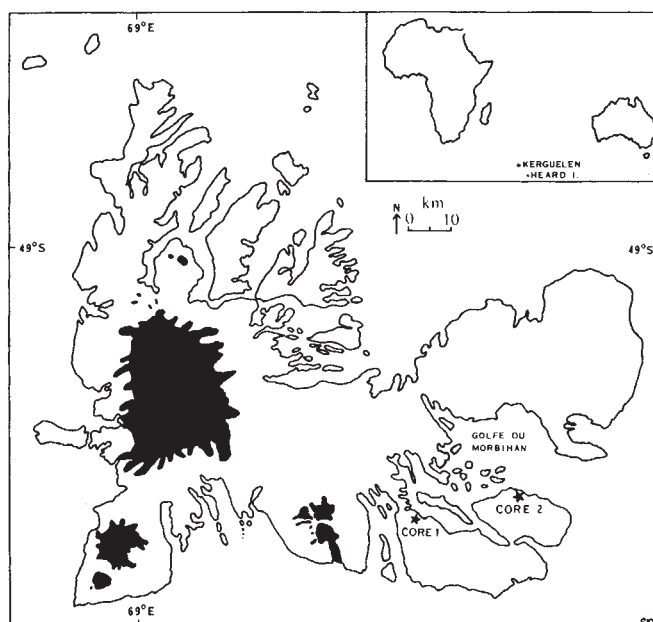


Fig. 1 Map of Kerguelen Islands indicating extent of present glaciers (black) and core sites. Inset shows position of islands in relation to Africa and Australia.

Kerguelen supports about thirty native species of vascular plants. Many are confined to a narrow range of habitats. The most extensive vegetation formation is the upland wind desert, dominated by the cushion plant, *Azorella selago* Hook f., which also occurs near sea level in drier exposed areas. Closed vegetation, dominated by *Acaena adscendens* Vahl, is abundant at lower elevations on the eastern portion of the island, but is rare or absent in the more rugged western regions⁴.

The ratio of *Azorella* to *Acaena* pollen has been used in a vegetation history of Marion and Prince Edward Islands⁵ as an indication of 'upland' versus 'lowland' conditions. Although the distribution of these plants on Kerguelen is not always correlated with elevation, this distinction is generally valid. We therefore regard the preponderance of *Azorella* pollen and lack of *Acaena* pollen in the lowest levels of both cores (Figs 2, 3) as evidence of cold 'upland' conditions. The rapid rise of *Acaena* pollen, at about 10,000 BP in core 2 and at a similar, but less accurately dated time in core 1, suggests a general warming trend. If there was a difference in the time of the onset of this trend at the two stations, it could be explained by the position of the retreating glaciers, which would have been nearer to the site of core 1. The subsequent wide fluctuations in the ratio of *Azorella* and *Acaena* pollen probably reflect local changes at the core sites. Pollen grains of Kerguelen grasses, although abundant, cannot be distinguished from one another and are of little value in determining climate. But pollen and spores of several rarer taxa help clarify the interpretation of the past 9,000 yr.

Lycopodium saururus Lam., *Blechnum penna-marina* (Poir.) Mitt., *Uncinia compacta* R. Br., and *Galium antarcticum* Hook f. are confined to protected habitats at elevations of less than 220 m (ref. 4). *Lycopodium saururus* and *Uncinia* are rare, and found only in the warmest situations. All of these species are apparently near their limit of cold tolerance on Kerguelen. Although widely distributed in the southern hemisphere, none occurs on Heard Island⁶, which lies 450 km south of Kerguelen and has a more polar climate. A significant rise of pollen or spores from more than one of these species would suggest a proliferation of 'lowland' conditions.

In both cores *Lycopodium saururus*, *Blechnum* and *Uncinia* first appear in noticeable quantities about 8,000 to 9,000 BP. The proportions rise rapidly, as do other, less important 'lowland' indicators such as *Lycopodium magellanicum* (Beauv.) Sw. (Figs 2 and 3). High proportions are maintained until about 6,000 BP, at which time *Galium* pollen rises. The spores and *Uncinia* quickly taper off after this point, but *Galium* remains common and reaches a secondary peak in core 2.

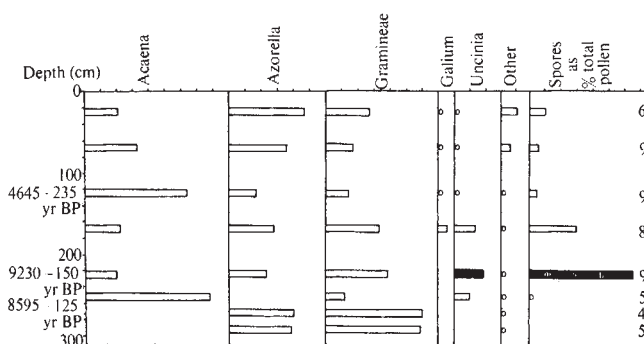


Fig. 2 Simplified pollen diagram of core 1 with ¹⁴C dates. Each division on left equals 20 cm, each division on top equals 10%. Each histogram represents an average of several counts (of 150 grains each), the number of which is shown on right. An open circle indicates 2% or less. Solid black histograms identify maximum % of indicator species. Symbols on spore histogram: m, *Lycopodium magellanicum*; s, *L. saururus*; b, *Blechnum penna-marina*.

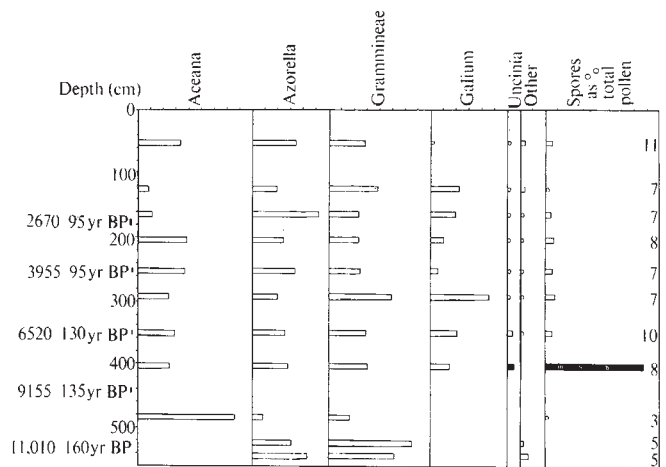


Fig. 3 Simplified pollen diagram of core 2. Symbols same as in Fig. 2.

The upper levels of both cores are marked by a continued low representation of the 'lowland' indicator species.

The following outline of climatic changes of Kerguelen can be constructed on the basis of our pollen analysis. A first warming trend began about 12,000 BP, culminating in a major glacial retreat by 10,000 BP. Thereafter, low temperatures prevailed for about 1,000 yr; most of the deglaciated areas supported a cold-tolerant vegetation consisting mainly of *Azorella* and grasses. *Acaena* became important as a major warming trend began. By 8,000 BP several cold-tolerant species had colonised the core sites. This apparent expansion of a vegetation containing a mixture of 'lowland' species suggests that the climate of Kerguelen at that time was considerably warmer than at present. *Blechnum* and *Uncinia* presently grow most luxuriantly in moist habitats, while *Galium* is usually found in dry habitats. As *Galium* pollen reached a peak later than the other 'lowland' species, possibly the latter part of the climatic optimum was drier. By 5,000 BP, the cold-tolerant species (partially excepting *Galium*) had declined to low levels, indicating that the warm period on Kerguelen was over. A similar history has been found in southern Chile, where the climatic optimum was followed by a glacial readvance about 4,500 BP (refs 1 and 7). In the northern hemisphere the climatic optimum tapered off more slowly and elevated temperatures often persisted until about 3,000 BP (refs 1 and 2).

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¹ Heusser, C. J., in *World Climate from 8000 to 0 B.C.* (edit. by Sawyer, J. S.), 124 (Royal Meteorological Society, 1966).

² Flint, R. F., *Glacial and Quaternary Geology* (Wiley, New York, 1971).

³ van Zinderen Bakker, E. M., sen., in *Palaeoecology of Africa and of the Surrounding Islands and Antarctica*, 5 (Balkema, Cape Town, 1969).

⁴ de la Rue, E. Aubert, *Comité National Français des Recherches Antarctiques*, No. 10, Biologie, 1, 1 (1964).

⁵ Schalke, H. J. W. G., and van Zinderen Bakker, E. M., sen., in *Marion and Prince Edward Islands* (edit. by van Zinderen Bakker, E. M., et al.), 89 (Balkema, Cape Town, 1971).

⁶ Lourteig, A., and Cour, P., *Comité National Français des Recherches Antarctiques*, No. 3, 63 (1963).

⁷ Mercer, J. H., *Am. J. Sci.*, 266, 91 (1968).

Interpretation of the Dirac Relationship between Fundamental Constants

SINCE the original relationship pointed out by Dirac^{1,2}, several dependent and independent numerical relations have been proposed. Dirac noticed that the ratio between the age of the Universe and the natural unit of time (which he took to be the time the light takes to cross an electron radius) is approximately equal to the ratio between the electrostatic and the gravitational force acting between two electrons. Here I propose a semi-empirical relationship which could throw some light on the Dirac expression:

$$m_e c^3 / (H e^2) \simeq e^2 / (G m_e^2) \quad (1)$$

where m_e and e are the electron mass and charge respectively, H is the Hubble constant, G is the gravitational constant and c is the speed of light. Whether m_e or m_p , the proton mass, should appear on the right hand side of equation (1) has long been regarded as irrelevant, since a few orders of magnitude would matter little in expressions of the order of 10^{40} . But here I shall need to be more accurate, and I write instead $m_e c^3 / (H e^2) = q e^2 / (G m_e^2)$, where q is an appropriate constant, and g comes from setting $R = gc/H$, with $g \leq 1$. Equivalently:

$$H = (g/q) (G m_e^2 c^3 / e^4) \quad (2)$$

I now propose that the Universe is endowed with an angular momentum L_u , which can be written as $L_u = (V_u/V_e) L_e$, where V_u and V_e represent the volumes of the Universe and of the electron respectively, and L_e is the angular momentum of the electron, namely $\hbar/2$. (I note, however, that so far there is no observational support for an overall rotation of the Universe^{3,4}.)

This conclusion was reached in a semi-empirical way, by considering a number of natural physical systems for which both the volume and the angular momentum could be estimated. They are represented in Fig. 1, and are broadly grouped into three "islands": (i) elementary particles, nuclei and atoms; (ii) planetary and stellar systems; and (iii) galactic systems. A rough proportionality $L \propto V$ appears from the graph, although there are exceptions. In fact neutron stars show an excess of angular momentum per unit volume and, more remarkably, a few nuclei and atoms (He in particular) have zero angular momentum. Elementary particles with $L=0$ seem to be unstable.

Another interesting consideration is that an irregular galaxy can be expected to possess a random angular momentum of the order of $(N_s)^{1/2} L_s$, where N_s is the number of stars, and L_s some typical value for the angular momentum of a star. Such a system would not disagree too much with my relationship. On the contrary, an uncollapsed cloud of ionized gas of the same mass could be expected to show a random angular momentum of the order of $(N_p)^{1/2} \hbar$, where N_p is the number of protons. Such an angular momentum would deviate considerably from $L \propto V$, but again these systems do not seem to be stable. The wide range covered both by V and L guarantees that if any relationship of the form $L \propto V^m$ exists at all, m is not much different from 1. I therefore conjecture that one can write:

$$L_u = (\hbar m_e^3 c^3 / 2 H^3 e^4) g^3 \quad (3)$$

where the radius of the Universe has been set at $R = gc/H$.

I now consider the simplest and most transparent cosmological model which embodies the previous features, namely an expanding, rigidly rotating Newtonian Universe. Models belonging to this category have been investigated by Heckmann and Schücking⁵, who obtained the relationship:

$$H^2/2 + \omega^2/3 - \Lambda c^2/6 = 4\pi G\rho/3 + h \quad (4)$$

where ω is the angular velocity, Λ is the cosmological constant (which may be positive, zero or negative), h is the energy constant, which I shall put equal to zero. I also write $\omega = kH$ (with $k = (1-g^2)^{1/2}/g$, in order to have $(\omega R)^2 + (HR)^2 = c^2$, which is necessary to explain redshifts of the order of 2, if g is small), and $\rho = 3pH^2/(4\pi G)$. Here p , k and g depend on the detailed model I choose.

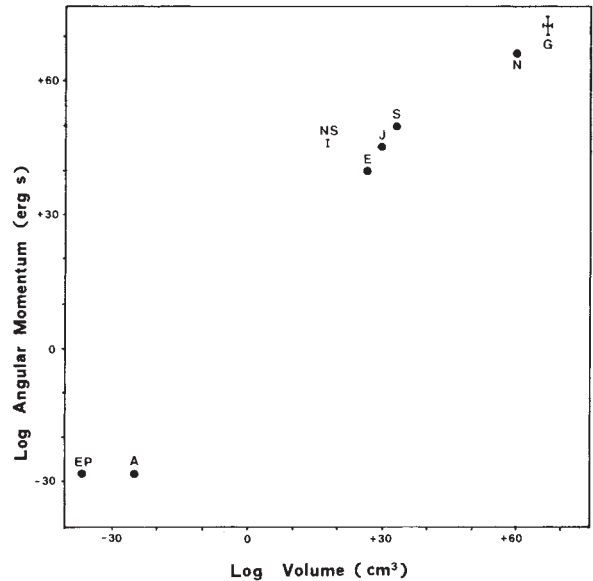


Fig. 1 Angular momentum as a function of volume of some natural systems. EP, Stable elementary particles; A, atoms; NS, neutron stars; E, Earth; J, Jupiter; S, Sun; N, galactic nucleus (M31); G, Galaxy.

The angular momentum of the foregoing model Universe is $L_u = I\omega$, where $I = 2MR^2/5$ is the moment of inertia. Substitution gives $L_u = 2pk g^2 c^3 / (5GH^2)$, which, divided by equation (3), affords a second relationship for H :

$$H = (5/4pk g^2) (\hbar m_e^3 c^4 G / e^4) \quad (5)$$

Division of equation (5) by equation (2) produces $\alpha \equiv e^2 / (\hbar c) = 5q/(4pg^3k)$.

I now consider possible choices for p and g , which are in principle unknown. The ratio q/g in equation (2) is better defined: for the conventional value $H = 100 \text{ km s}^{-1} \text{ Mpc}^{-1}$, $q/g = 1/125.4$. Recent determinations propose a lower value for H , although matters are not yet defined. I shall investigate two models which might be aesthetically more acceptable.

(a) Equipartition model, with $\Lambda = 0$. In this case $\omega = (3/2)^{1/2} H$, $g = (4/13)^{1/2}$, $p = 1$, with the consequence that $\alpha = 3.32 q/g$. For the above value of H one obtains $\alpha = 1/38$. A very similar value is obtained if one sets $\Lambda = 0$, $g = k = 0.786$, which require $p = 0.705$, and $\alpha = 3.65 q/g \simeq 1/34$.

On the whole this is not far from a fair agreement with the value $\alpha = 1/137$.

(b) I can also construct an *ad hoc* model, by proposing $q/g = 2\alpha$, which would correspond to the more modern value $H = 54 \text{ km s}^{-1} \text{ Mpc}^{-1}$. The somewhat disturbing factor of 2 automatically comes in if I take $R = c/(2H)$, namely $g = 1/2$. With this assumption $p = 10.3^{-1}$, which corresponds to a density of matter $\rho = 6.31 \cdot 10^{-29} \text{ g cm}^{-3}$. The requirement $g = 1/2$ produces $\Lambda = -25.6 H^2/c^2$. This choice of parameters does not correspond to a Lemaitre model, but to a closed universe.

Although I do not support any of these particular models, I think that equations (3) and (1) in the framework of an expanding, rigidly rotating Newtonian Universe are strictly related to each other, and equation (3), being semi-empirical, is perhaps richer in physical content and therefore preferable to equation (1). In fact, it seems that a much better expression of equation (1) is $m_e c^3 / (2He^2) = \alpha e^2 / (Gm_e^2)$. I note that, by substituting another "natural" unit of length in the place of the classical radius of the electron, such as the Compton wavelength of the electron or the Bohr radius of the hydrogen atom, I merely change the power of α in front of the right hand side to α^2 and to α^3 respectively. This removes the arbitrariness of the "natural" unit of length adopted by Dirac, and at the same time suggests that a "more fundamental" unit of length is $e^4 / (\hbar m_e c^3) = 2.05 \times 10^{-15} \text{ cm}$, for which I would have the power α^0 .

At present, I do not suggest any interpretation for this unit of length.

I thank Drs H. Horstman and G. Palumbo for discussions.
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¹ Dirac, P. A. M., *Nature*, **139**, 323 (1937).

² Dirac, P. A. M., *Proc. R. Soc.*, **A165**, 199 (1938).

³ Hawking, S., *Mon. Not. R. astr. Soc.*, **142**, 129 (1969).

⁴ Wolfe, A. M., *Astrophys. J. Lett.*, **159**, L61 (1970).

⁵ Heckmann, O., and Shücking, E., *Helv. Phys. Acta, Suppl.*, **4**, 114 (1956).

BIOLOGICAL SCIENCES

Serological Relationship of Swine Vesicular Disease Virus and Coxsackie B5 Virus

THE recent outbreak of swine vesicular disease (SVD) in England¹ has focused attention on the characteristics of the causative virus. The virus belongs to the enterovirus subgroup of the picornaviruses² and the strains isolated from the first outbreak in Italy in 1966² and later in Hong Kong in 1971³ and England in 1972¹ are serologically related. We describe here the relationship of this porcine enterovirus to a human enterovirus of man, and speculate that the virus might have been transferred from man to swine.

Swine vesicular disease virus (SVDV) is fatal to newborn mice^{2,3}. This property defines the Coxsackie group of the enteroviruses. A possible relationship was sought by testing the ability of Coxsackie virus antisera to neutralize SVDV. The SVDV used for most observations was the Hong Kong 36/71 strain (SVDV-HK) supplied by the Animal Virus Research Institute, Pirbright, England. Two other strains of SVDV—the English strain UKG 27/72 (SVDV-UKG), and Italian strain, Italy 1/66 (SVDV-IT)—supplied by the same institute were also used.

The method of testing the neutralization of SVDV by Coxsackie virus antiserum was by using the enterovirus immune horse serum pools prepared according to the Lim Benyesh-Melnick scheme⁴. These sera were provided by the National Institute of Allergy and Infectious Diseases, Bethesda, Maryland. The neutralization patterns of viruses by these eight pools identify the serotypes of forty-two different enteroviruses of man.

For the neutralization test, two-fold dilutions of each serum pool were prepared in the wells of microtitre plates (Linbro FB-TC). A suspension containing 300 TCID₅₀ of SVDV

was added to each dilution and, after incubation for 30 min at 37° C, tissue culture growth medium containing 5,000 cells of a pig kidney cell line, PK-15, was added. After incubation at 37° C for 4 d, the neutralization pattern was determined by staining the plates with a solution of 0.1% crystal violet in 10% formalin. Serum dilutions that neutralized the virus had complete cell monolayers, whereas the non-neutralized dilutions were cell-free. The neutralizing titre was the highest dilution of serum protecting the cells. Table 1 shows that SVDV-HK was neutralized by serum pools C and E which identifies the Coxsackie virus B5 serotype⁴.

This identity was verified by intracranial inoculation of a 1/20 dilution of each serum pool mixed with 1,000 TCID₅₀ of SVDV-HK in the newborn mice of one litter for each serum-virus mixture. After 5 d, all mice inoculated with pool C or E serum-virus mixtures were alive, while those inoculated with other combinations died (Table 2).

Table 2 Neutralization of Swine Vesicular Disease Virus in Newborn Mice by Enterovirus Horse Serum Typing Pools

Pool designation *	Mouse survival
A	0/9†
B	0/10
C	9/9
D	0/9
E	9/9
F	0/10
G	0/10
H	0/10
Control	0/19

* See text.

† Number alive/number inoculated 5 d after inoculation.

The Faulkner strain of Coxsackie B5 virus was obtained from the American Type Culture Collection and tested for its neutralization by a serum obtained from a pig 56 d after recovery from infection by SVDV-HK. In the same test, SVDV-HK and the pool E serum containing Coxsackie B5 antiserum were included. Serial dilutions of each virus were mixed with a 1/320 dilution of the sera and PK-15 cells added. The neutralizing index was computed as the difference in log titre of each virus with the antiserum and with normal swine serum. Table 3 shows that significant cross neutralization was observed.

Table 3 Neutralization Index of Swine Vesicular Disease Virus (Hong Kong) and Coxsackie Virus B5 by Homologous and Heterologous Antisera

Antiserum (1/320)	Swine vesicular disease	Coxsackie B5
Swine vesicular disease	4.3 *	5.2
Coxsackie B5 (pool E)†	2.3	4.4

* Log difference of virus titrated in presence of antiserum and normal serum.

† See text.

Titration of SVDV-IT and SVDV-UKG with each of the typing pools also showed that significant neutralization only occurred with pools C and E. A serological relationship of the SVDV strains and Coxsackie B5 virus was thus established.

Sera from United States swine were surveyed for evidence of this disease. Lots of twenty sera each were obtained from swine in New Jersey, North Carolina, Massachusetts and California, and seventy sera from Virginia. None of these neutralized SVDV-HK in tissue culture. Sera from swine recovered from Teschen disease and foot-and-mouth disease also failed to neutralize SVDV-HK, whereas swine recovered from SVD had virus-neutralizing titres of 1/200 to 1/10,000. Serum from guinea pigs which neutralized 300 TCID₅₀ of SVDV-HK at a dilution of 1/512 did not neutralize any of

Table 1 Neutralization of Swine Vesicular Disease Virus in Tissue Culture by Enterovirus Horse Serum Typing Pools

Pool designation *	Serum neutralizing endpoint
A	—†
B	—
C	640
D	—
E	640
F	—
G	—
H	—

* See text.

† Reciprocal titre less than 20.

six common swine enteroviruses—E-1, E-2, ECPOs 2, 5, 6, 8—at any serum dilution.

A 30 kg pig was inoculated intravenously with 5 ml of Cocksackie B5 virus that had been grown in the pig kidney cell line, IBRS-2⁹. Another was fed 25 ml of the same material and a third was inoculated intranasally with 5 ml daily for 5 d. None showed clinical signs of disease but all developed significant virus neutralizing antibody titres against Cocksackie B5 virus and SVDV. This indicated that Cocksackie B5 virus replication took place in the exposed swine. The virus used had eleven passages in mouse brain and four in monkey kidney tissue culture cells before growth in the IBRS-2 cells. It is reasonable to speculate that virus with a different passage history might have caused disease in the exposed pigs.

The reciprocal situation of testing the susceptibility of man to infection with SVDV is not readily investigated. The relationship of SVDV to a human virus can, however, be shown by the neutralizing of SVDV by human sera. Significant neutralization of SVDV-HK by twenty-two of twenty-four human serum samples was found. Fourteen were from workers in a laboratory where SVDV was present but they did not have significantly higher titres than those from non-laboratory-associated persons. Determination of whether this neutralization was due to previous infection with SVDV or Cocksackie B5 virus depends on the development of a test to distinguish differences between the virus strains; however, the absence of SVDV neutralizing antibody in serum samples from normal swine as well as absence of clinical signs of the disease supports the contention that the virus-neutralizing antibody in human sera was due to past infection with Cocksackie B5 virus.

The serological relationship between SVDV and Cocksackie B5 virus suggests the possibility of transmission of a virus between the two species. The absence of SVDV neutralizing activity in normal swine sera indicates that SVDV may well be a new enterovirus of the porcine species and the reciprocal evidence of SVDV neutralizing antibody in man implies a contemporary experience in humans with a virus very closely related to SVDV. The development of SVDV neutralizing antibody in the pigs exposed to Cocksackie B5 virus indicates that infection of swine by this virus can occur. We may be observing the development of a new disease of pigs, with good indication that the origin of the causative agent was man.

I thank J. B. Brooksby, director of the Animal Virus Research Institute, Pirbright, for the viruses and IBRS-2 cell line and for consultation with his staff, in particular F. Brown and R. Burrows. I also thank E. Appelt, N. Shuot and W. Doroski for technical assistance, and P. D. McKercher, Plum Island Animal Disease Center, for useful discussions. The swine enteroviruses were supplied by A. H. Dardiri of the same laboratory.

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Antigenic Differences between Isolates of Swine Vesicular Disease Virus and their Relationship to Cocksackie B5 Virus

THERE have been several outbreaks of a vesicular disease in swine in which the causative agent was shown to be an enterovirus¹⁻³. The first occurred in Italy in 1966¹ and this was followed by an outbreak in Hong Kong in 1971². A more serious and widespread outbreak occurred in the United Kingdom during the winter of 1972-73³ and several other cases were reported in Austria, France, Italy and Poland during the same period. Further cases also occurred in the United Kingdom in June 1973. From an epidemiological point of view it was important to know whether the same strain of virus had caused the disease in the different countries. Differences between some isolates could be demonstrated by complement fixation tests using specific antisera prepared in guinea pigs (Arrowsmith, A., personal communication) and by cross-neutralization tests using sera from convalescent swine (ref. 2 and R. B. Mann, J. A., and Goodridge, D., unpublished). We show here that unequivocal antigenic differences between some of the isolates can be demonstrated by immunodiffusion tests.

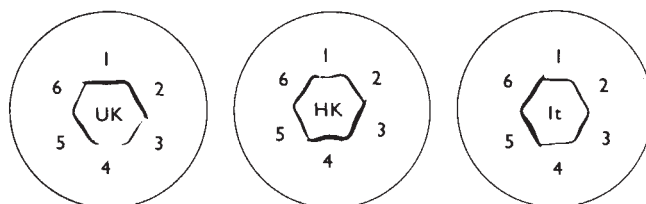


Fig. 1 Immunodiffusion tests of three isolates of SVDV with each specific convalescent swine serum. 1 and 2, United Kingdom 1972 virus; 3 and 4, Hong Kong 1971 virus; 5 and 6, Italy 1966 virus; UK, United Kingdom 1972 serum; HK, Hong Kong 1971 serum; It, Italy 1966 serum.

Purified preparations of the viruses were allowed to diffuse through 0.75% (w/v) agarose towards either serum from convalescent swine which had been infected with the different isolates or hyperimmune serum from guinea pigs which had been inoculated with purified preparations of the different viruses. A precipitin line common to all the isolates was observed with each serum but in addition a specific spur line was obtained with each virus and its homologous serum when the viruses in adjacent wells were antigenically different. Figure 1 shows the reactions obtained with the viruses isolated from the outbreaks in Italy in 1966, Hong Kong in 1971 and the United Kingdom in the winter of 1972-73. The spur lines were clearly visible when observed directly but they were difficult to record photographically. To obtain a record of the reactions, autoradiograms of the precipitin patterns were made, using radioactively labelled viruses in the immunodiffusion tests.

Similar tests were made with the viruses isolated from the outbreaks in 1972-73 in Austria, France, Italy, Poland and the United Kingdom. With the exception of the French isolate, no antigenic differences could be demonstrated between these viruses. A typical test, in which the Austrian and United Kingdom isolates were compared with the French isolate, is shown in Fig. 2. The French virus was also antigenically different from the Italy 1966 and Hong Kong 1971 isolates. These results suggest that the viruses causing the recent outbreaks in Austria, Italy, Poland and the United Kingdom are antigenically similar to each other but different from the French isolate and from those occurring in Italy in 1966 and Hong Kong in 1971.

Isolates from infected swine on eight farms in different parts of the United Kingdom were compared by immuno-

¹ Dawe, P. S., Forman, A. J., and Smale, C. J., *Nature*, **241**, 540 (1973).

² Nardelli, L., Lodetti, E., Gualandi, G. F., Burrows, R., Goodridge, D., Brown, F., and Cartwright, B., *Nature*, **219**, 1275 (1968).

³ Mowat, G. N., Darbyshire, J. H., and Huntley, J. F., *Vet. Rec.*, **90**, 618 (1972).

⁴ Schmidt, M. J., Melnick, J. L., Wenner, H. A., Ho, H. H., and Burkhardt, M. A., *Bull. Wld. Hlth. Org.*, **45**, 317 (1971).

⁵ Dardiri, A. H., *Can. J. comp. Med.*, **32**, 416 (1968).

⁶ Castro, M. P. de, and Pisani, C. D., *Arg. Inst. Biol. (Sao Paulo)*, **34**, 295 (1967).

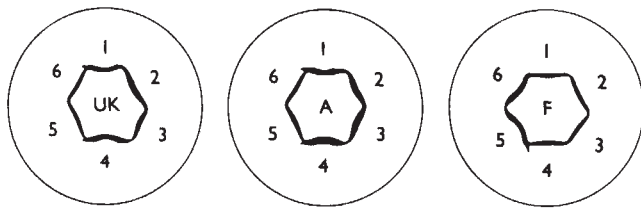


Fig. 2 Immunodiffusion tests of three isolates of SVDV with each specific convalescent swine serum. 1 and 2, United Kingdom 1972 virus; 3 and 4, Austria 1973 virus; 5 and 6, France 1973 virus; UK, United Kingdom 1972 serum; A, Austria 1973 serum; F, France 1973 serum.

diffusion tests. With the viruses isolated during the winter outbreak no antigenic differences could be found. The virus isolated from a recent outbreak (June 1973) in Wrexham, Denbighshire, was, however, found to be antigenically different from those obtained during the winter outbreak. This new isolate was also different from those obtained from Austria, France, Hong Kong, Italy (1966 and 1972) and Poland.

Graves⁴ has shown recently that the Italy 1966, Hong Kong 1971 and United Kingdom 1972-73 isolates are neutralized by Cocksackie B5 antiserum. Antiserum against the Hong Kong isolate of swine vesicular disease virus (SVDV) also neutralized Cocksackie B5 virus. However, Cocksackie B5 virus could be clearly distinguished by immunodiffusion tests from all the SVDV isolates we have examined. A precipitin line common to Cocksackie B5 virus and the SVDV isolates was obtained but in addition a specific spur was obtained with each virus and its homologous antiserum. The antigenic difference between Cocksackie B5 virus and the United Kingdom 1972-73 and Italy 1966 isolates of SVDV is shown in Fig. 3.

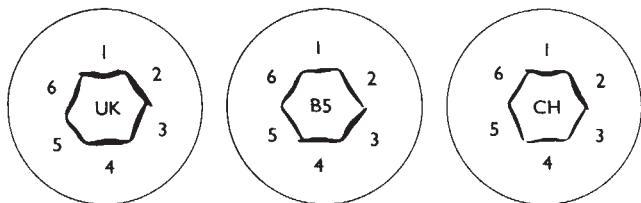


Fig. 3 Immunodiffusion tests of two isolates of SVDV and Cocksackie B5 virus with an SVDV convalescent swine serum and serum from a patient convalescing from aseptic meningitis. 1 and 2, United Kingdom 1972 virus; 3 and 4, Cocksackie B5 virus; 5 and 6, Italy 1966 virus; UK, United Kingdom 1972 serum; B5, Cocksackie B5 antiserum; CH, serum from patient convalescing from aseptic meningitis.

This technique for differentiating between SVDV and Cocksackie B5 has also proved useful in examining the cause of some illnesses occurring among workers at this institute during the winter of 1972-73. These workers experienced symptoms similar to those which occur in Cocksackie virus infections. All had been engaged in laboratory or animal experiments with SVDV and serum samples taken from them late after infection contained high levels of neutralizing activity against this virus (R. B., Mann, J. A., and Goodridge, D., unpublished). The close serological relationship between Cocksackie B5 virus and SVDV demonstrated by Graves⁴ raised the question whether these illnesses had been caused by infection with Cocksackie B5 virus or with SVDV. Immunodiffusion tests on the sera with high levels of neutralizing activity gave specific spur lines with SVDV, suggesting that the illnesses in these cases had probably been caused by this virus. An example with the serum from one of the patients is shown in Fig. 3.

These results demonstrate the use of the immunodiffusion test in comparing strains of virus in outbreaks of swine vesicular disease, with its obvious application to epidemiological studies, and indicate that there are several antigenically distinct strains of the virus. The experiments also suggest that SVDV may cause illness in humans, similar to that found in Cocksackie virus infections.

We thank Dr J. H. Graves, Plum Island Animal Disease Laboratory, New York, USA, for discussions and for making available his results on SVDV and Cocksackie B5 virus before publication. We also thank Dr D. R. Gamble, Public Health Laboratory, Epsom, Surrey, for providing the Cocksackie B5 virus and antiserum and the staff of the World Reference Laboratory of this Institute for the SVDV isolates.

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- ¹ Nardelli, L., Lodetti, E., Gualandi, G. L., Burrows, R., Goodridge, D., Brown, F., and Cartwright, B., *Nature*, **219**, 1275 (1968).
- ² Mowat, G. N., Darbyshire, J. H., and Huntley, J. F., *Vet. Rec.*, **90**, 618 (1972).
- ³ Dawe, P. S., Forman, A. J., and Smale, C. J., *Nature*, **241**, 540 (1973).
- ⁴ Graves, J. H., *Nature*, **245**, 314 (1973).

Analysis of Virus Replication in Ageing Human Fibroblast Cultures

SINCE Swim and Parker¹ and Hayflick and Moorhead² documented the limited life span of diploid human fibroblasts in cell culture, many investigators have used these cells as possible models of cellular senescence. Most normal human fibroblasts exhibit marked cellular changes and eventually cease growth after fifty to seventy cell divisions. Hayflick³ and Martin *et al.*⁴ demonstrated an inverse relationship between the life span of cultured human diploid fibroblasts and the age of the donor. Furthermore, fibroblasts from children with premature ageing diseases have a markedly reduced potential for cell division⁴. Many investigators have studied human fibroblasts to determine whether their finite life span is related to ageing, and to determine the mechanism of 'senescence' in these cells⁵. There is no firm evidence, however, that fibroblast 'ageing' or limited fibroblast division potential is necessarily involved in the tissue and organ changes associated with ageing of individuals. Many types of cells which are obviously important in ageing of the whole organism undergo little or no division in adults (for example, neurones), and others which divide rapidly (for example, intestinal epithelium) have not been shown to behave as fibroblasts do, either in culture or in the organism.

Orgel⁶ suggested that accumulating errors at the level of translation could eventually lead to an error catastrophe, and that such effects might be involved in ageing cells. Holliday and Tarrant⁷ and Lewis and Tarrant⁸ demonstrated that diploid human fibroblasts accumulate heat-labile enzymes during the final stages of their life span in culture. They showed that the RNA base analogue 5-fluorouracil induced premature senescence in these cells, and again altered enzyme was observed. Lewis and Tarrant⁸ found that the ratio of active enzyme to cross-reacting material changed considerably as ageing cells approached death, and that the cells simultaneously lost the ability to discriminate between methionine and its analogue ethionine (the relative incorporation of ethionine increased markedly

with senescence). These results are compatible with the error catastrophe theory, but as Lewis and Tarrant⁸ point out, they do not conclusively support it since the accumulation of altered protein in ageing cells may not necessarily be a cause of ageing and death.

We have used virus infection to assess further the state of senescent human fibroblasts. The introduction of viral genes into ageing cells allows determination of their ability to replicate and translate viral genetic information independently of the mutational status of the cell DNA (except as cellular mutations may have affected the translational machinery). If the ageing fibroblasts are synthesizing a large percentage of abnormal proteins, then viral replication should be affected (due to altered viral replicases) and viral maturation should be defective since some altered proteins would be likely to interact in the assembly process involving hundreds or thousands of structural proteins per virion. Hayflick and Moorhead² showed that non-senescent diploid human fibroblasts are susceptible to various RNA and DNA viruses, and we report here the effect of senescence on susceptibility to three viruses.

Table 1 Virus Yields from Senescent and Control (Low Passage) WI38 Human Fibroblasts

Cell passage level	Cell No. yield (PFU)	Total virus yield (PFU)	Virus yield per cell (PFU per cell)
Vesicular stomatitis virus			
Passage 21 (control)	1×10^6	1.6×10^{10}	16,000
Passage 59 (senescent)	1×10^6	2×10^{10}	20,000
Passage 24 (control)	2×10^5	3×10^9	15,000
Passage 51 (senescent)	2×10^5	2.5×10^9	12,500
Passage 32 (control)	1×10^6	2×10^{10}	20,000
Passage 60 (senescent)	1×10^6	3×10^{10}	30,000
Type 1 poliovirus			
Passage 23 (control)	1×10^6	1.8×10^9	1,800
Passage 61 (senescent)	1×10^6	2.6×10^9	2,600
Passage 22 (control)	1×10^6	3.9×10^9	2,000
Passage 59 (senescent)	1×10^6	2.4×10^9	2,400
Herpes simplex virus type 1			
Passage 23 (control)	1×10^7	5×10^{10}	5,000
Passage 60 (senescent)	1×10^7	5×10^{10}	5,000
Passage 32 (control)	1×10^6	2×10^9	2,000
Passage 61 (senescent)	1×10^6	1.6×10^9	1,600

High passage senescent cell cultures were from different stocks of the WI38 cell and some became senescent at later passage numbers than others. All senescent cells (above passage 50) used in these experiments were terminally senescent (cell division had ceased in nearly all cells). Control cells at low passages were rapidly replicating cultures. VSV plaque assays¹³ were carried out on BHK₂₁ hamster cells, poliovirus plaque assays on HeLa cells, and herpesvirus assays on a diploid human fibroblast cell strain established in this laboratory. In all of the above experiments but one, control and senescent cells were infected at a multiplicity of 50–100 PFU per cell. In the second VSV experiment (passages 24 and 51) a low multiplicity of infection was used (0.01 PFU per cell). In all cases virus yields were determined when cytopathology was maximal (all cells showed total cytopathology in control and senescent cultures by about 24 h following VSV and poliovirus infection and by 40 h following herpesvirus infection). Low multiplicity infection of senescent cells with herpesvirus gave very slowly progressing cytopathology and lower yields. The protein content per cell of control and senescent fibroblasts was approximately the same.

Table 1 demonstrates that high passage senescent WI38 fibroblasts and low passage control cells were equally susceptible to poliovirus, to vesicular stomatitis virus (VSV) and to *Herpes simplex* virus type 1. Even though the senescent cells used were at the final possible passage before death, and all cells had ceased dividing, the virus yields per cell were equal to those in the non-ageing low passage control cell cultures. Viral cytopathology and death were

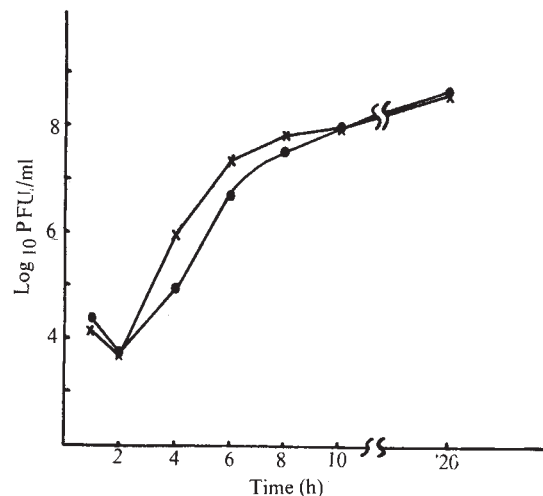


Fig. 1 Kinetics of production of infectious VSV by senescent cells and low passage control cells of WI38 strain diploid human fibroblasts. Senescent and control cells (2×10^5) were infected with Indiana serotype VSV at a multiplicity of 100, and 0.05 ml samples of the medium were withdrawn at indicated times following washing and incubation in 3 ml of fresh medium at 37° C. (All infectious virus was released into the medium since VSV matures by budding to the extracellular space from the plasma membrane.) ×, Senescent WI38 cells at terminal passage (passage 51); ●, control non-senescent WI38 cells at passage 24.

complete in all cells in both high passage and low passage cultures. Table 2 shows that senescent cells produced virus of approximately the same specific infectivity as did low passage control cells. Senescent cells did not seem to be making an unusual number of inactive virus particles. This ability of ageing cells to support normal yields was surprising since these were terminal passage cultures in which all cells exhibited advanced signs of senescent degeneration (cytoplasmic granularity, highly branched cytoplasmic processes and so on).

Table 2 Specific Infectivity of Vesicular Stomatitis Virus Grown in Senescent Cells and in Control Low Passage Cells

Cells in which the virus pool was grown	Specific infectivity (PFU per mg protein)
Passage 23 (control WI38 cells)	2.1×10^{11}
Passage 61 (senescent WI38 cells)	1.9×10^{11}

Senescent and control cells were infected at a multiplicity of 1 and virus yields were collected and purified after 20 h at 37° C. Purification involved differential centrifugation, agarose gel chromatography and sucrose gradient rate zonal centrifugation. The amount of pure virus protein recovered was related to the total PFU determined from a small sample of the unpurified virus suspension, not from the purified virus which aggregates and loses considerable infectivity after the first concentration step. No correction was made for virus loss during purification, and it is assumed that purification losses were similar for senescent and control cells. Plaque assays were performed on BHK₂₁ cells¹³.

Figure 1 compares the kinetics of production of VSV by senescent cells and low passage control cells. It can be seen that mature infectious virus was produced more rapidly by ageing fibroblasts than by rapidly growing control cultures. It is clear that the senescent cells are at least as efficient in replicating and assembling VSV as non-senescent controls.

It seemed possible that in spite of the efficiency with which infectious virus was produced, the virions from senescent cells might contain a significant proportion of abnormal structural proteins which would render them more thermolabile than virions produced by normal cells. Figure 2 demonstrates that this is not so. The infectivity of virions produced in senescent cells was thermally

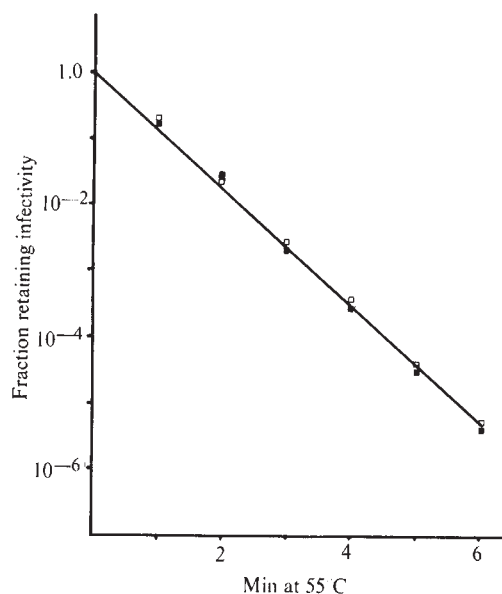


Fig. 2 Comparison of the kinetics of thermal inactivation at 55° C of VSV grown in senescent and low passage WI38 cells. Virus yields from both sources were collected 20 h after infection, adjusted to equal concentrations in Eagle's minimum essential medium containing 10% calf serum and heated in small samples for indicated times in this medium. □, Virus from terminal passage senescent (passage 51) WI38 cells; ■, virus from control non-senescent WI38 cells at passage 24.

inactivated at 55° C at a rate identical with the inactivation of virions produced by low passage cells. At 55° C, pseudo-first-order inactivation kinetics were obtained with virus from both non-ageing and senescent cells (Fig. 2). At 50° C, virus from low passage and from high passage cells again showed identical inactivation kinetics, but a complex multi-component curve was seen in both cases (data not presented). VSV is a complex membrane virus containing thousands of proteins some of which assemble into structures with helical and icosahedral or hexagonal symmetry⁹. If a significant proportion of abnormal viral structural proteins are synthesized in the senescent cells they must be excluded from the viral assembly process. Similar thermal stability was observed for poliovirus grown in senescent cells and control cells.

If generalised error catastrophe is involved in senescence of cells, errors in protein synthesis could lead to mutagenesis through synthesis of altered polymerases and the mutations could in turn lead to further errors in translation as outlined by Holliday and Tarrant. Therefore, we have compared the mutation rates of poliovirus grown in senescent cells and in low passage control cells. Poliovirus induces a replicase in infected cells¹⁰ and, if the replicases synthesized in senescent cells include a significant percentage of abnormal molecules, the mutation rate might be altered. Growth of wild type poliovirus is inhibited by 10⁻³ M guanidine^{11,12} and spontaneous mutation to guanidine resistance can be measured accurately.

Table 3 shows that the spontaneous rate of mutation to guanidine resistance is not significantly altered during replication in senescent cells. Virus yields from senescent cells exhibited the same low level of mutants for this character as were found in yields from low passage cells. It appears that highly mutagenic replicase molecules are not being synthesized at significant levels in infected senescent cells.

These results are not in accord with a situation in which generalised translation error is occurring, but they do not disprove error catastrophe in which translational errors are not generalised. For example, it is possible that errors might be restricted to certain unusual codons. Alternatively,

viruses might have evolved so that they can replicate and mature efficiently in spite of translational error. Altered viral proteins might be relatively unstable or unable to compete with unaltered replicase molecules or with structural proteins in replication and assembly. The most obvious explanation for the difference between the present results and those of Holliday and Tarrant⁷ and Lewis and Tarrant⁸ is based on the possibility, which they pointed out, that the lowered specific activity of cellular enzymes might be due to accumulation of inactive enzyme in dead or dying cells. Since viral proteins are all newly synthesized in senescent cells, the presence of 'old' viral proteins was not a factor in the study reported here.

Table 3 Comparison of Mutation Rates (to Guanidine Resistance) of Poliovirus Grown in Senescent Cells and Control Low Passage Cells

Cells in which the polio-virus pool was grown*	Total virus yield (PFU)	Yield of guanidine-resistant mutants (PFU)	Fraction of mutants in yield
Passage 23 WI38 (control)	1.8 × 10 ⁹	8 × 10 ⁴	4.4 × 10 ⁻⁵
Passage 61 WI38 (terminal senescence)	2.6 × 10 ⁹	7 × 10 ⁴	2.7 × 10 ⁻⁵

* Senescent or control WI38 fibroblast monolayer cultures were infected at a multiplicity of 50 by adsorption of type 1 poliovirus wild type to 1 × 10⁶ cells for 30 min. Infected cells were washed thoroughly and incubated at 37° C in Eagle's minimum essential medium plus 10% calf serum. No guanidine was added. The virus yields were collected 18 h after infection, and the total yields from each culture were determined by plaque assay on HeLa cells under Eagle's medium containing 0.4% agarose. The yield of guanidine resistant mutants from each culture was determined by plaque assay on HeLa cells under Eagle's medium containing 0.4% agarose and 1 mM guanidine hydrochloride. Plaques were stained and counted after incubation at 37° C for 48 h (ref. 13).

Further studies are needed before the error catastrophe theory can be proved or disproved. Obviously, careful quantitation of the synthesis, the specific activity and the turnover rates of a virus-induced enzyme could provide considerable insight into the synthetic capabilities of senescent fibroblasts. In all such investigations it must be remembered that fibroblast senescence in culture may or may not be relevant to the cellular changes undergone by fibroblasts and other cell types during ageing of the whole organism.

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- Swim, H. E., and Parker, R. F., *Am. J. Hyg.*, **66**, 235 (1957).
- Hayflick, L., and Moorhead, P. S., *Exptl Cell Res.*, **25**, 585 (1961).
- Hayflick, L., *Exptl Cell Res.*, **37**, 614 (1965).
- Martin, G., Sprague, C., and Epstein, C., *Lab. Invest.*, **23**, 86 (1970).
- Holečková, E., and Cristofalo, V. J., eds., *Ageing in Cell and Tissue Culture* (Plenum, New York, 1970).
- Orgel, L. E., *Proc. natn. Acad. Sci. U.S.A.*, **49**, 517 (1963).
- Holliday, R., and Tarrant, G. M., *Nature*, **238**, 26 (1972).
- Lewis, C. M., and Tarrant, G. M., *Nature*, **239**, 316 (1972).
- Cartwright, B., Smale, C. J., Brown, F., and Hull, R., *J. Virol.*, **10**, 256 (1972).

- ¹⁰ Baltimore, D., Eggars, H. J., Franklin, R. M., and Tamm, I., *Proc. natn. Acad. Sci. U.S.A.*, **49**, 843 (1963).
¹¹ Loddo, B., *Boll. Soc. ital. biol. Sper.*, **37**, 395 (1961).
¹² Crowther, D., and Melnick, J. L., *Virology*, **15**, 65 (1961).
¹³ Holland, J. J., and McLaren, L. C., *J. Bact.*, **78**, 596 (1959).

Effect of Neuraminidase Pretreatment on the Susceptibility of Normal and Transformed Mammalian Cells to Bovine Enterovirus 261

TAYLOR *et al.* reported regression of ascites and solid tumours in mice inoculated with bovine enterovirus 1 (BEV-1)¹ without any observable pathological effect on the animals. The basis of the oncolytic specificity was associated with the adsorption spectrum of the virus^{2,3}. The specificity was also expressed *in vitro* since virus transformed and oncogenic cells were susceptible to BEV-1, in contrast to cells of untransformed lines and of primary cultures, which were resistant⁴.

Our results demonstrate that bovine enterovirus 261 (BEV-261)⁵ has an oncolytic specificity similar to that of BEV-1. However, normal human cells were lysed after treatment with the virus when the infected cultures were overlaid with agar. Data on the susceptibility of neuraminidase-treated normal and transformed cells to BEV-261 are discussed here.

Strain L clone 929 mouse fibroblasts were purchased from Grand Island Biological Company, New York. 3T3 cells were supplied by Dr J. Robb, University of California, San Diego. SV40-transformed 3T3 cells were originally obtained from Dr Helene Smith, University of California, Berkeley. Moloney sarcoma virus-transformed Balb/c mouse cells were obtained from Dr J. Massicot of the National Cancer Institute. HeLa, WI-38, L-132 (human embryonic lung), BHK-21 (clone 13), and BS-C-1 monkey kidney cells were purchased from the American Type Culture Collection. HFM human diploid skin fibroblasts were initiated in culture by G. D. S.

Cell monolayers were cultured in 75 cm² plastic flasks (Falcon) each containing 15–20 ml minimal essential medium (MEM) supplemented with 10% heat-inactivated (56° C, 30 min) foetal calf serum and in an atmosphere of 5% carbon dioxide and air. Confluent cultures of transformed and untransformed cells were trypsinized (0.1% trypsin) and split 1:8 and 1:4 respectively at 7-d intervals.

Madin-Darby bovine kidney (MDBK) cells cultivated in MEM were used to grow high titre lysates of BEV-261. The cells were infected, and virus was collected and assayed by the plaque technique in MDBK cells as described by Taylor *et al.*².

To determine the susceptibility of the cultured cells to infection with BEV-261, confluent cultures were trypsinized and the cell suspensions were inoculated into each of a series of 60×15 mm tissue culture dishes (Falcon). After 1–2 d, the medium was removed and replicate cultures inoculated with 0.1 ml of three dilutions of virus; that is, 10⁻³, 10⁻⁴, 10⁻⁵ (original virus stock containing 1.8×10⁷ PFU ml⁻¹). After incubation for 1 h at 37° C, the monolayers were washed three times with 3–5 ml pre-warmed phosphate-buffered saline (PBS) and replaced with 5 ml medium containing 10% GG-F newborn calf serum. At daily intervals after infection, all plates were examined microscopically for evidence of cytopathic effects (CPE). When CPE was observed, the cultures were rinsed with saline and the cells fixed with ethanol: glacial acetic acid (3:1) and stained with Harris haematoxylin solution. The remaining cultures were transferred twice for further examination of the cells for CPE. These cultures

were then rinsed, cells were fixed and stained. At least four cultures were used for each virus dilution and for controls.

Sialic acid is known to influence the adsorption of several viruses to cells⁶⁻⁸. To determine whether it participates in BEV-261-cell interaction, confluent monolayers of transformed strain L and MDBK cells and normal HFM human skin cells cultured in 60×15 mm plates were treated with *Vibrio cholera* neuraminidase (VCN), as described by Helgeland⁷, before exposure to BEV-261. Cells treated with either virus or VCN, heated VCN (56° C, 30 min) and untreated cultures were controls. All cultures were overlaid with agar⁷ and plaques were counted after 24–72 h.

Table 1 Response of Various Cell Types to Treatment with BEV-261

Cell type	Characteristics	<i>In vitro</i> CPE
3T3	Contact inhibited mouse fibroblasts (stable)	—
3T3 SV40*	Viral transformed (not stable)	+
Balb/c MSV†	Viral transformed mouse fibroblasts (not stable)	+
L cells	Continuous cell line (not stable)	+
HFM	Human diploid skin fibroblasts (stable)	—
WI-38	Human diploid lung fibroblasts (not fully stable)	±
L-132	Human embryonic lung (not stable)	+
HeLa	Human cervical carcinoma (not stable)	+
BHK-21 (clone 13)	Hamster kidney fibroblasts (not fully stable)	+
BS-C-1	Continuous cell line (not stable)	+

* SV40, Simian virus 40.

† MSV, Moloney sarcoma virus.

+, Cytopathic effect; —, no cytopathic effect.

Table 1 presents data on the CPE of BEV-261 on cultured cells. CPE usually developed within 24–48 h at all virus dilutions. The data indicate that all transformed cells were susceptible to BEV-261. "Normal" 3T3 and HFM cells were resistant; however, as shown in Table 2, plaques could be observed in infected HFM cells through use of agar overlay technique. Similar to the results of Sedmak *et al.*⁴, WI-38 cells were slightly susceptible to BEV-261.

The data in Table 2 illustrate that pretreatment of HFM cells with VCN (2 or 5 U) for 2 or 24 h rendered them completely unsusceptible to BEV-261 as measured by inhibition of plaque formation. Similar treatment had no effect on the susceptibility of MDBK or L cells to the virus. However, pretreatment of MDBK cells with 50 U VCN for 24 h resulted in an average 60% decrease in PFU per monolayer. Data for L cells are given in estimated percentage monolayer destruction because, under agar, virus-infected cells were only partially lysed and the plaques could not be easily counted. Untreated cultures and cultures treated with VCN only were normal during the experiments. As expected, HFM cells treated with heat-inactivated VCN were as susceptible to BEV-261 as untreated cultures.

Table 2 also indicates that MDBK and L cells are more susceptible to BEV-261 than HFM cells; little or no viral activity could be observed in HFM cultures at the 10⁻⁴⁻⁵ dilution of virus (virus stock contained 1.8×10⁷ PFU/ml).

The *in vitro* oncolytic specificity of BEV-261 is very similar to that of BEV-1⁴; that is, transformed cells were susceptible and normal cells appeared resistant to the virus. However, CPE could be observed in normal human HFM cells when the cultures were treated with higher concentrations of virus than were used for HeLa and L-132 cells, and when the infected cultures were overlaid with agar. Presumably, the agar overlay served to localize the virus to enable infection. Complete lysis of infected HFM cultures occurred within 5–6 d. Although not tested, other normal cell types may have been susceptible to BEV-261 under these conditions.

These results seem important when considering the use of enteroviruses for therapy of human cancer as suggested by Sedmak *et al.*⁴ Treatment of cancer patients, particularly those with well progressed tumours, would probably require high concentrations of virus. We have shown that BEV-261 can destroy normal human cells *in vitro*. If, in similar *in vitro* conditions, other bovine enteroviruses have the same effect, then treatment of cancer patients with these entities could lead to destruction of normal tissues and other undesirable complications. The use of low doses of enterovirus to destroy residual tumour cells after treatment by surgical, radiological or chemical methods might be more practical.

Table 2 Response of HFM, MDBK and L Cells to Infection with BEV-261 after Treatment with Neuraminidase

Cell type	Virus dilution	Treatment		PFU per monolayer			Mean
		U of neuraminidase per ml	Hours				
HFM	10 ^{-3.3}	2.0	2	0	1	0	0.33
		5.0	2	0	0	0	0
		2.0	24	0	0	0	0
		5.0	24	0	0	0	0
		No treatment		47	58	52	53
		* HIVCN 5.0		42	41	63	48
				Percentage monolayer destruction (estimated)			
MDBK	10 ^{-4.5}	2.0	2	111	94	103	103
		5.0	2	85	96	99	93
		2.0	24	89	110	110	103
		5.0	24	78	97	99	91
		50.0	24	32	41	44	39
		No treatment		98	97	102	99
L	10 ^{-4.5}	2.0	2	50	40	30	40
		5.0	2	30	20	40	30
		2.0	24	60	25	30	38
		5.0	24	30	40	30	33
		No treatment		40	30	30	33

Vibrio cholera. Neuraminidase obtained from Behringwerke AG had an activity of 500 U ml⁻¹. One neuraminidase unit was the amount of enzyme required to release 1 µg of sialic acid from human α₁-acid glycoprotein in sodium acetate-acetic acid buffer (pH 5.5) in 15 min at 37° C. The preparation was claimed to be free of aldolase, lecithinase C and proteolytic enzymes.

* HIVCN, heat-inactivated (56° C, 30 min) *Vibrio cholera* neuraminidase (5 U ml⁻¹).

The data from preliminary experiments with VCN-treated HFM cells and MDBK cells indicate that sialic acid participates in the adsorption of BEV-261 to both normal and transformed cells. The observation that larger quantities of VCN were required to remove virus receptor from MDBK cells suggests that they possess more sialic acid than normal HFM cells. This agrees with several reports indicating that transformed cells contain more sialic acid than do normal cells, and may explain why they are more susceptible to BEV-261. Studies are in progress to define further the cell receptor for BEV-261.

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¹ Moll, T., and Davis, A. D., *Am. J. vet. Res.*, 20, 27 (1959).

- ² Taylor, M. W., Davidson, J. N., Land, C., and Wall, R., *J. natn. Cancer Inst.*, 44, 515 (1970).
- ³ Taylor, M. W., Cordell, B., Souhrada, M., and Prather, S., *Proc. natn. Acad. Sci. U.S.A.*, 68, 836 (1971).
- ⁴ Sedmak, G. V., Taylor, M. W., Mealey, J., and Chen, T. T., *Nature new Biol.*, 238, 7 (1972).
- ⁵ Moll, T., and Ulrich, M. I., *Am. J. vet. Res.*, 24, 545 (1963).
- ⁶ Tsvethova, I. V., *Biokhimiya*, 32, 994 (1967).
- ⁷ Helgeland, K., *Acta Path. Microbiol. scand.*, 68, 439 (1966).
- ⁸ Boulanger, P. A., Houdret, N., Scharfman, A., and Lemay, P., *J. gen. Virol.*, 16, 429 (1972).

Mutagenesis of Mouse Myeloma Cells with 'Melphalan'

TECHNIQUES have been developed to study the potential mutagenic effects of various drugs on mouse myeloma cells. Cells from mouse plasmacytomas have been adapted to continuous culture, cloned in soft agar, and the clones have been overlaid with antiserum specific for the immunoglobulin chains. The immunoglobulin secreted by the myeloma cells precipitates with the antibody in the agar surrounding the clones. Clones which have lost the ability to secrete immunoglobulin remain unstained by antigen-antibody precipitates and can be recovered and scored as variants¹. Mouse myeloma cells which produce IgG spontaneously lose the ability to produce heavy chains and produce light chains at a rate of 1.1 × 10⁻³ per cell per generation. The incidence of variants is not increased by treatment with the alkylating agent, ethyl methane sulphonate. Nitrosoguanidine causes a two-to-three-fold increase in variants while the acridine half mustard, ICR-191, which is effective in producing frame shift mutations in microorganisms², produces a twenty-fold increase in variants³.

The phenylalanine mustard, 'Melphalan' (*p*-[di-(2 chloro-ethyl)amino]-L-phenylalanine) is frequently used in the treatment of human multiple myeloma. Following treatment with the drug, patients have been reported to show an increase in the amount of light chain produced, an apparent change in the structure of the paraprotein itself, or complete loss of paraprotein synthesis⁴. Because these changes are similar to those which we have seen in cultured mouse myeloma cells, the effect of 'Melphalan' on the mouse myeloma cells has been examined.

Mouse myeloma cells derived from the MPC-11 tumour, which produces IgG_{2b}, were maintained in continuous suspension culture in Dulbecco's modified Eagle's medium (Grand Island Biological Co.) supplemented with glutamine, non-essential amino acids, penicillin, streptomycin, and 20% heat inactivated horse serum. 'Melphalan' (Burroughs Wellcome) was dissolved in propylene glycol at 0.2 to 1.0 mg ml⁻¹ and diluted in growth medium just before treatment of the cells. A freshly recloned line of MPC-11 cells was used to minimize the background of spontaneous variants. Cells growing logarithmically at a concentration of 2–6 × 10⁵ cells ml⁻¹ were incubated in the presence of the drug for 24 h at 37° C in 5% CO₂. Control cells were treated with similar concentrations of propylene glycol. Cells were removed from the drug by centrifugation, incubated in fresh medium for an additional 24 h, and then cloned in soft 'Agarose' on rat embryo fibroblast feeders as described previously⁵. Cells (1,500–6,000) were cloned in separate dishes and after several days, when individual clones were easily identified, the dishes were overlaid with antiserum specific for the MPC-11 heavy chain. Clones secreting heavy chains containing molecules were surrounded and partially obscured by an antigen-antibody precipitate⁵. Clones which had either lost the ability to secrete heavy chains or were producing an antigenically deficient heavy chain were not surrounded by a visible precipitate and were scored as variants. Such unstained clones were recovered, grown up to mass culture, and their intracellular

and secreted immunoglobulins were further characterized by double diffusion in agar (Ouchterlony). Selected variants were incubated with either ^{14}C - or ^3H -amino acids; their cytoplasmic extracts and secretions were immunologically precipitated and analysed on sodium dodecyl sulphate (SDS)-containing acrylamide gels⁶.

Table 1 Incidence of Unstained Colonies after *in vitro* Treatment of Plasmacytoma Cells with Various Concentrations of 'Melphalan'

'Melphalan' g ml ⁻¹	Cell killing	No. of clones	No. of un- stained clones	Unstained clones (%)
0	0	3,777	17	0.45
2×10^{-7}	68%	2,336	31	1.33 *
4×10^{-7}	72%	5,926	100	1.69 *
6×10^{-7}	84%	2,961	55	1.86 *
8×10^{-7}	91%	1,298	31	2.39 *
ICR-191 1.10^{-6} g ml ⁻¹	57%	1,284	40	3.1 *

Cell killing was calculated by multiplying the cell survival at the time of plating by the relative cloning efficiency (ratio of the cloning efficiency in the 'Melphalan' treated dishes over the cloning efficiency in the control dishes).

* $P < 0.001$ (χ^2 test).

When the cultured mouse myeloma cells were treated with doses of 'Melphalan' which killed 68–91% of the cells, a significant increase in the incidence of unstained clones was observed (Table 1). Cell killing and the incidence of variants increased with increasing amounts of the drug. In conditions where 91% of the cells were killed, there was a six-fold increase in variants and more than 2% of the surviving clones were unstained. In the same experiment, treatment with a dose of ICR-191 which killed 57% of the cells caused an incidence of variants of 3.1% (Table 1).

To ascertain that the high incidence of variants was not due to selective killing of the wild type cells, light chain producing variants obtained from the non-mutagenized control dishes were grown to mass culture and their susceptibility to killing by 'Melphalan' was compared with the total parent culture. No difference was detected.

Twenty presumptive variants were recovered from the plates and analysed by double diffusion in agar with a variety of antisera. Sixteen did not contain detectable amounts of intracellular heavy chain when analysed with either antibody made against denatured MPC-11 heavy chains or antibody against the Fc region of the MPC-11 paraprotein. All sixteen clones synthesized and secreted light chains. The radioactively labelled cytoplasmic and secreted immunoglobulins of some of these presumptive light chain producers were analysed on SDS-containing acrylamide gels. Heavy chains were not detected. Based on previous experiments, heavy chains would have been detected if they were synthesized at 1/50–1/100th the rate present in the parent⁵.

In contrast, the remaining four 'Melphalan' variants contained intracellular immunoglobulins which reacted with antibody made against denatured MPC-11 heavy chains. Ouchterlony analysis using a variety of antisera showed that the immunoglobulins produced by these four variants lacked antigenic determinants present in the parent protein.

The SDS gel analysis of the immunoglobulins produced by one of these variants is shown in Fig. 1. Proteins from the parental clone were used as markers. The migration of molecules in the SDS acrylamide gel system is a function principally of their size, so the gels show that a variety of immunoglobulin molecules of abnormal size were produced and assembled by the variant. When the intracellular immunoglobulins of the parent and the variant were reduced, alkylated and analysed together on the same gels, the variant heavy chain was distinctly smaller than that of the parent (41,000 as compared with 55,000). The variant L chain was slightly smaller than the parent (inset of Fig. 1). Preliminary peptide maps of the variant H chain revealed that

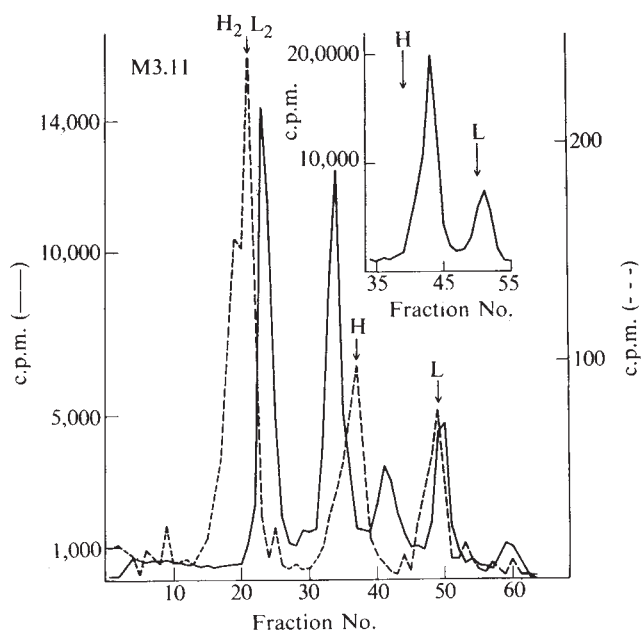


Fig. 1 SDS acrylamide gel electrophoresis of immunoglobulins produced by a 'Melphalan' variant (—). The cells were labelled for 10 min with ^{14}C -valine, threonine and leucine. Cytoplasmic extracts were prepared by lysis with NP 40 in the presence of 0.06 M iodoacetamide, and immune precipitated by a rabbit and MPC-11 protein serum and a sheep and rabbit gamma globulin serum. The immune precipitates were dissolved in 2% SDS and co-electrophoresed in 0.1% SDS, 5% acrylamide gels with purified H_2L_2 , H and L polypeptide chains from the parent clone (---). Inset: The same material after reduction and alkylation. The arrows indicate the position of the parent H and L chains.

it lacks eight or nine of the thirty-five chymotryptic peptides found in the parent. Preliminary SDS acrylamide gel analysis of the four variants producing defective heavy chains revealed significant differences amongst all of them.

Each of these variants was recloned after 5 weeks in culture. Twenty to twenty-five subclones of each have been examined by double diffusion in agar and all of the clones maintained the serological characteristics of the variant. No revertants were found when 2,000 subclones of each of the variants were examined by overlaying them with the antiserum used in the original plate assay. The variants have continued to be stable serologically and electrophoretically for the 4–5 months since they were isolated.

These studies indicate that 'Melphalan' is very effective in inducing stable and heritable changes in immunoglobulin production in mouse myeloma cells. The characteristics of both the light chain and defective heavy chain producing variants and their very high incidence are similar to those which we have observed with ICR-191³. The high spontaneous "mutation" rates and incidence of immunoglobulin variants following mutagenesis are not a general property of these cells since drug resistant variants arise at rates which are orders of magnitude lower than those reported here (ref. 3 and unpublished data). Either genetic or epigenetic mechanisms could be responsible for the frequency of both the spontaneous and drug induced variants.

It is likely, however, that the 'Melphalan' variants producing the abnormally sized heavy chains are genetic in origin in that they reflect a change in chromosomal DNA. Further structural and genetic characterization of these variants will be necessary to determine whether their high incidence is a result of one or more of the unusual aspects of the genetic control of immunoglobulin.

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¹ Coffino, P., and Scharff, M. D., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 219 (1971).

² Drake, J. W., *The Molecular Basis of Mutations* (Holden-Day, San Francisco, 1970).

³ Baumal, R., Birshtein, B. K., Coffino, P., and Scharff, M. D., *Science*, N.Y. (in the press).

⁴ Hobbs, J. R., *Br. med. J.*, **2**, 67 (1971).

⁵ Coffino, P., Baumal, R., Laskov, R., and Scharff, M. D., *J. cell. Physiol.*, **79**, 429 (1972).

⁶ Laskov, R., and Scharff, M. D., *J. exp. Med.*, **131**, 515 (1970).

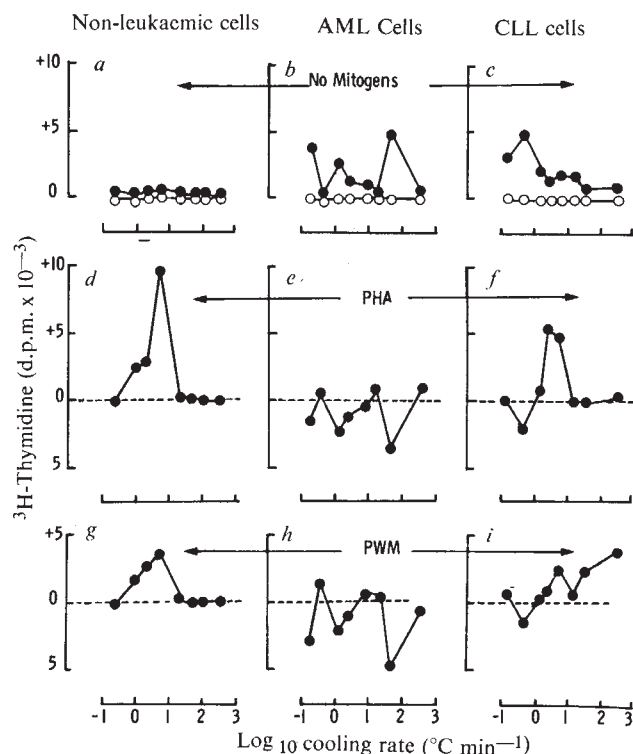


Fig. 1 Effect of cooling rate on ³H-thymidine uptake (d.p.m.) by thawed cells from a normal individual, an AML patient and a CLL patient on the fourth day of culture. *a-c*, Without mitogen in the presence of allogeneic AML serum (●) or non-leukaemic serum (○). *d-i*, Changes in d.p.m. in the presence of mitogens during culture with allogeneic AML serum (●).

Selection of Leukaemic Cell Populations by Freezing and Thawing

THE co-existence of normal and abnormal cell populations in the peripheral blood of leukaemic patients may explain the variations in the reactivity to mitogens (discussed by Ling¹). One example is the low or delayed response of cells from chronic lymphocytic leukaemic (CLL) patients to phytohaemagglutinin (PHA) in comparison with normal cells. The variations presumably result from a combination of the response of the underlying normal population to mitogens and the different responses (inhibition, no effect or stimulation) of the abnormal cells. Analysis of abnormal cell populations and their interactions, together with the study of serum factors, is hindered by the heterogeneity of leukaemic cell suspensions. We have now investigated the differential survival of leukaemic and normal cells from leukaemic blood following freezing and thawing using controlled cooling conditions. Previously we established the cooling rate at which normal human peripheral lymphocytes are recovered optimally using dimethylsulphoxide (DMSO) as a protective additive^{2,3}. Lymphocytes activated by mitogen or specific antigen before freezing required different cooling rates for optimal recovery. We show here that cooling rates both faster and slower than those required for the recovery of normal lymphocytes permit the differential survival of abnormal cells from leukaemic blood and that this technique may clarify the study of serum factors.

Cell samples were obtained from three untreated acute myeloid leukaemic (AML) patients using an IBM cell separator, from defibrinated peripheral blood of four untreated CLL patients and from six normal donors. Cells obtained by gelatine separation were cooled at different rates to -60°C in 0.5 ml aliquots containing 2×10^7 mononuclear cells in 12×35 mm polypropylene tubes (Sterilin) in the presence of DMSO (5% v/v) in Dulbecco's medium and stored in liquid nitrogen. As our aim was to produce selective killing, serum (itself cryoprotective) was omitted from the freezing mixture and, after thawing the samples, they were rapidly diluted with Dulbecco's medium before removal of the DMSO³. The viability of thawed lymphocytes assessed by trypan blue dye exclusion tests showed little correlation with their capacity to respond to mitogens

*in vitro*³. For assessment in culture, therefore, a constant dilution factor for cells recovered at each rate was used². Assuming 30% survival of cells, 0.2 ml microplate cultures contained 2×10^5 cells in Dulbecco's medium in the presence of 10% leukaemic or normal serum, with or without mitogen: PHA (Burroughs Wellcome MR10) or pokeweed mitogen (PWM) (Difco)^{2,3}. ³H-thymidine (0.2 μCi , 150 $\mu\text{Ci mmol}^{-1}$) (Amersham) was added 24 h before collection on the fourth day of culture and the uptake into the acid-precipitable fraction of the cells was measured by liquid scintillation counting^{2,3}.

Peripheral blood lymphocytes from normal individuals recovered from samples frozen at different cooling rates showed minimal incorporation of ³H-thymidine in the presence of autochthonous, normal allogeneic or leukaemic serum without mitogens (Fig. 1*a*). Responses of these control cells to PHA and PWM were recovered in the presence of each serum after cooling the cells at $2-7^{\circ}\text{C min}^{-1}$ (Fig. 1*d* and *g*).

This was in sharp contrast to the picture of recovery obtained with cells taken from the peripheral blood of AML patients. Cells recovered after cooling at rates both faster and slower than those found to allow recovery of normal peripheral blood cells incorporated ³H-thymidine when cultured with allogeneic or autochthonous AML serum in the absence of mitogens (Figs 1*b* and 2*a*). In the presence of normal sera, however, this uptake of ³H-thymidine was never observed (Fig. 1*b* and *c*). Thus in acute myeloid leukaemia there seems to be at least two populations of abnormal cells, distinguishable by alteration of the cooling rate, which proliferate in the presence of AML sera but not when cultured with normal sera. The relative proportions of each population (assessed by uptake of ³H-thymidine) varied between individuals. A clear distinction between populations of cells incorporating ³H-thymidine was shown when cells frozen at $1.2^{\circ}\text{C min}^{-1}$ were thawed and refrozen over a range of cooling

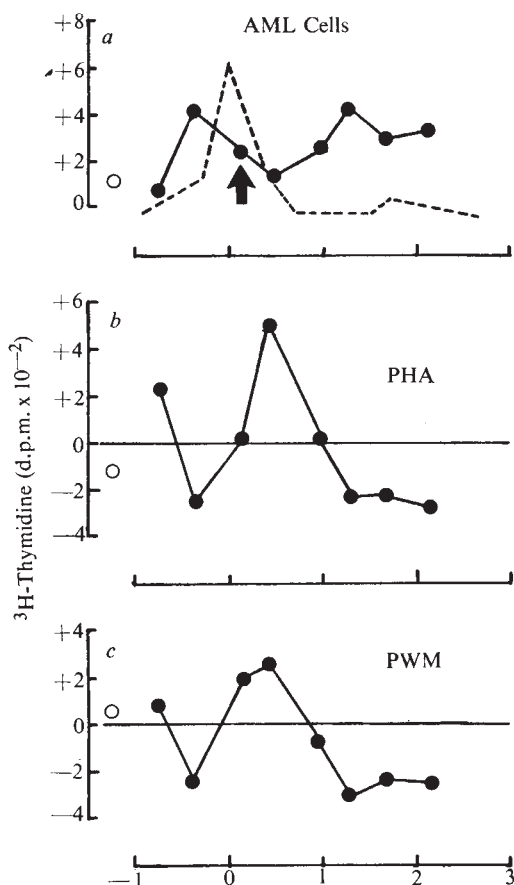


Fig. 2 Effect of cooling rate on ^3H -thymidine uptake (d.p.m.) by thawed cells from an AML on the fourth day of culture with autochthonous serum. *a*, Without mitogen ($\bullet$); *b*–*c*, changes in d.p.m. due to the presence of mitogens during culture ($\bullet$). For comparison, the response of aliquots of unfrozen cells ($\circ$) is also shown. Cells thawed after cooling at $1.2^\circ \text{C min}^{-1}$ (arrow) were refrozen using a range of cooling rates and, on thawing, concentrated and cultured without mitogens (dashed line).

rates. Most cells synthesizing DNA in leukaemic serum in the absence of mitogens were recovered at the cooling rate used for the first freeze; little or no activity was recovered at the faster rates (Fig. 2*a*). It is not yet clear whether the serum effects that we are observing are due to stimulating factors present in AML sera or to an inhibitory or controlling action of normal sera. Uptake of ^3H -thymidine in the presence of these AML sera (without mitogens) was also found with cells from CLL patients (Fig. 1*c*); again, the incorporation was not seen with normal sera.

With both leukaemias, peripheral cells cooled at the rate known to be optimal for the recovery of small lymphocytes from normal donors showed some characteristics of normal cells: they had low spontaneous ^3H -thymidine incorporation and were usually stimulated by both PHA and PWM (Figs 1 and 2). The response to these mitogens, and by implication the proportion of normal cells present, varied between patients. Addition of mitogens to cultures of thawed cells with high spontaneous ^3H -thymidine uptake often caused an inhibition of incorporation suggesting the preferential recovery of abnormal cells (Figs 1*e*, *h* and 2*b*, *c*).

Using cells from CLL patients, PWM responses were also recovered after cooling at fast rates (Fig. 1*i*). These cells were not responsive to PHA (Fig. 1*f*) suggesting that in these patients a population of abnormal B lymphocytes may have been recovered selectively using fast cooling rates. In contrast, in AML, PHA-responsive cells were

recovered after cooling at the slower rates (Fig. 2*b*) implying an abnormality of the PHA-responsive cell population.

The separation of populations of cells is also shown by the comparison between the ^3H -thymidine uptake of the mixed (unfrozen) populations with those of the cells cooled at the different rates (Fig. 2).

These procedures provide a basis for the study of the interactions between serum factors and discrete cell populations in leukaemia. In addition, the *in vitro* proliferative patterns observed with thawed cells cooled at different rates may allow an additional classification of leukaemia and provide a system for monitoring the course of the disease. Discrepancies between reports^{4,5} on mixed lymphocyte reactions between remission cells and stored acute-stage leukaemia cells may owe something to the selective properties of the cooling conditions, for it is clear that storing leukaemic cells in liquid nitrogen using a single cooling schedule can alter the spectrum of cells recovered.

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- ¹ Ling, N. R., *Lymphocyte Stimulation* (North Holland, Amsterdam, 1968).
- ² Knight, S. C., Farrant, J., and Morris, G. J., *Nature new Biol.*, **239**, 88 (1972).
- ³ Farrant, J., Knight, S. C., and Morris, G. J., *Cryobiology*, **9**, 516 (1972).
- ⁴ Fridman, W. H., and Kourilsky, F. M., *Nature*, **224**, 277 (1969).
- ⁵ Rudolph, R. H., Mickelson, E., and Thomas, E. D., *J. clin. Invest.*, **49**, 2271 (1970).

Cholecystokinin elicits Satiety in Rats with Open Gastric Fistulas

WITHIN 10 min of starting to eat, a rat stops eating¹, grooms for a short period, then usually sleeps. We define this behavioural sequence as satiety: its physiological basis is unknown. Beaumont² presented the first evidence that the presence of food in the gut is important in generating satiety signals. In 1949, Janowitz and Grossman³ confirmed Pavlov's observation⁴ that oesophagostomized dogs, in which no food reached the stomach or intestines, ate prolonged meals.

We have investigated the physiological mechanisms causing satiety by studying sham feeding in rats with chronic gastric fistulas. We report evidence that the polypeptide hormone cholecystokinin (CCK), which is released when food enters the small intestine⁵, may be a satiety signal in the rat.

All experiments were performed on adult male Sprague-Dawley rats weighing 350–450 g. Rats were housed in individual cages and kept in an artificial 12 h light cycle. They were maintained on a balanced liquid diet (No. 116EC, General Biochemicals, Chagrin Falls, Ohio) diluted 1:1 with water. Tap water was also available.

Six rats were equipped with chronic gastric fistulas under Equi-thesin anaesthesia (Jensen-Salsbury, Kansas City, Missouri). A stainless steel cannula (length 18 mm, inner diameter 6 mm) was sutured into the rumen of the stomach along the greater curvature. The external end of the cannula was closed with a removable screw. The animals were allowed to recover for one week before testing was begun; during this week, solid food pellets (Purina Rat Chow) and water were provided.

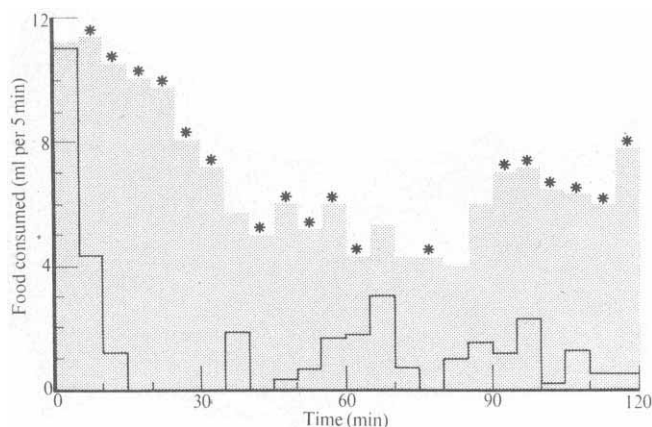


Fig. 1 Liquid diet consumed by six rats with gastric fistulas open or closed. After 17 h of deprivation, six rats with gastric fistulas closed or open were offered liquid diet diluted 1 : 3 with tap water. Tap water was also available throughout the test period. Lines represent mean intake of liquid diet when fistulas were closed; shaded bars represent mean intake when fistulas were open. ★, Intake when fistulas were open was significantly greater ($P < 0.05$, matched-pair t test, two-tailed) than when fistulas were closed.

The rats were deprived of food, but not of water, for 17 h from 1800 h to 1100 h. Thirty min before food presentation the gastric fistulas were opened and flushed with 5 ml of 0.15 M NaCl. Flexible stainless steel extension tubes⁶ were screwed into the gastric cannulas; they extended through a channel 10 mm wide which ran the length of each animal's cage. The extension tubes could be either closed or left open. The rats were presented with liquid diet diluted 1 : 3 with water in graduated tubes, and measurements of consumption were made at 5-min intervals for 2 h. At the end of the test period, the volume of fluid collected in pans beneath each cage was measured. The extension tubes were removed, the gastric cannulas closed and the rats were given liquid diet diluted 1 : 1 with tap water for the rest of the day.

The rats were tested with gastric fistulas closed for five consecutive days. On the sixth day the fistulas were left open during the test period. Food consumption on days 5 and 6 was compared for each 5-min period using a matched pair t test, each animal serving as its own control.

The rats ate more when the fistulas were open than when they were closed, and the difference was striking (Fig. 1). On day 5, with gastric fistulas closed, the rats took an initial meal during the first 15 min; then they stopped eating completely for 20 min and appeared to sleep. On day 6, with gastric fistulas open, the rats never became satiated; they ate nearly continuously throughout the test period and appeared to be aroused constantly. (Although rats never became satiated, there was a decrease in the rate of ingestion during the 25–85 min interval of the test period. This decrease may represent inhibitory signals subthreshold for satiety.) The results demonstrate that food in the gut is necessary to elicit satiety in the rat; since rats did not become satiated when the gastric fistulas were opened for the first time, satiety must be an inhibitory reflex and not a learned response under our experimental conditions.

Gut hormones, released by food entering the stomach and small intestines, may be satiety signals. We reported recently that intraperitoneal injection of a partially purified preparation of cholecystokinin (CCK), but not of secretin, reduced feeding in the intact rat^{7,8}. This suppression of feeding was dose-related for both solid and liquid foods and it did not appear to act by making rats sick. The suppression was a property of the CCK molecule in the extract, because identical doses of the synthetic C-terminal octapeptide of CCK (which possesses the full biological activity of the complete molecule⁹) produced identical results.

We tested the ability of CCK and of the synthetic octapeptide to reduce sham feeding in rats with open gastric fistulas. Eight rats equipped with chronic gastric fistulas were placed on the daily deprivation schedule described above. They were tested with gastric fistulas open. After the liquid diet had been presented, and the animals were eating rapidly, they were injected intraperitoneally with 1 ml of one of the following treatments on different days: (1) partially purified CCK (10% w/w, GIH Research Unit, Karolinska Institutet, Stockholm, Sweden) in doses of 10, 17, 20, 40, 80 or 160 Ivy dog units kg^{-1} dissolved in 0.15 M NaCl; (2) synthetic C-terminal octapeptide of CCK (SQ 19,844) in doses of 20, 40 or 80 Ivy dog units kg^{-1} dissolved in 0.15 M NaCl; (3) the desulphated synthetic C-terminal octapeptide of CCK (SQ 19,265), a preparation which has little of the activity of the sulphated molecule^{10,11}, in a dose of 3.6 $\mu\text{g kg}^{-1}$, a weight equivalent to 80 Ivy dog units kg^{-1} ; or (4) 0.15 M NaCl. Each animal served as its own control, and statistical comparisons were made with a matched-pair t test.

Both partially purified CCK and the synthetic C-terminal octapeptide of CCK suppressed sham feeding in rats with gastric fistulas open during the 30 min following injection (Fig. 2). The extent of the suppression depended on dose.

The sulphate group on the tyrosyl residue in the seventh position from the C terminal is necessary for the satiety action of CCK, because the desulphated synthetic C-terminal octapeptide of CCK had no effect on feeding (Fig. 2). The lowest dose of CCK tested (10 units kg^{-1}) significantly reduced feeding. This dose is approximately four times that required to decrease consumption of liquid diet in the intact rat⁸. This difference is not surprising: if endogenous CCK is acting as a satiety signal, then the threshold dose of exogenous CCK required to inhibit feeding in intact rats, which release endogenous CCK, should be less than the threshold dose of exogenous CCK in rats with open gastric fistulas, which probably do not release endogenous CCK.

When rats stopped eating after CCK and octapeptide injections they appeared satiated: there was a short period of grooming followed by a marked decrease in activity and

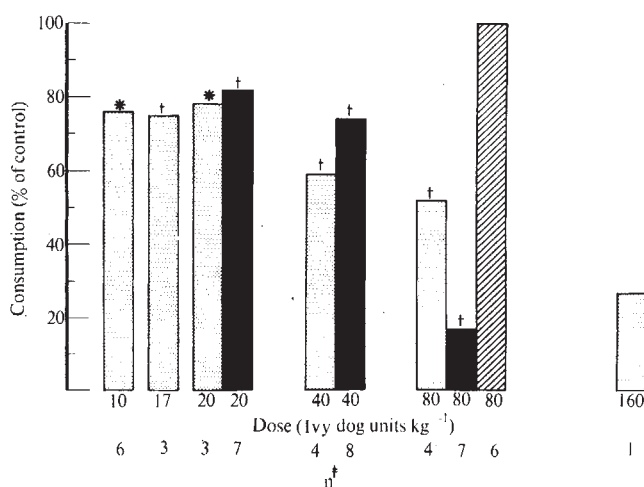


Fig. 2 Effects of cholecystokinin, octapeptide and desulphated octapeptide on consumption of liquid diet during 30 min following injection in rats with open gastric fistulas. After 17 h of deprivation, rats with open gastric fistulas were offered liquid diet diluted 1 : 3 with tap water. Tap water was also available throughout the test period. Consumption of liquid diet was measured during the 30 min following intraperitoneal injections of either CCK (light columns) synthetic C-terminal octapeptide of CCK (dark columns) or desulphated synthetic C-terminal octapeptide of CCK (hatched column), each dissolved in 0.15 M NaCl and expressed as a percentage of the amount eaten after intraperitoneal injection of 0.15 M NaCl alone. Mean control intakes for all tests ranged from 50.0 ml to 73.3 ml. Statistically significant differences from control. ★ $P < 0.05$. † $P < 0.02$ (matched-pair t test, two-tailed). ‡ n = number of rats.

apparent sleep. This was the same behavioural sequence previously observed in intact rats following a meal; there was no sign of physical distress.

We have not yet measured the plasma levels of CCK following a spontaneously taken meal in the rat, and until we can demonstrate that the amount of exogenous CCK required to elicit satiety is within the physiological range, we cannot state whether the satiety effect described is a physiological function of the CCK released by such a meal. Maximal pancreatic response to CCK has, however, been reported in anaesthetized rats given doses of 8–10 Ivy dog units kg^{-1} intravenously¹², and maximal gastric acid output to CCK and octapeptide of CCK has been reported in conscious rats given doses of 8–16 Ivy dog units kg^{-1} intravenously¹³. The lowest effect dose of CCK reported in the present experiments is within the range of these studies of gastrointestinal function.

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- ¹ Kissileff, H. R., *Physiol. Behav.*, **5**, 163 (1970).
- ² Beaumont, W., *Experiments and Observations on the Gastric Juice and the Physiology of Digestion*, 208 (Dover Publications, New York, 1959).
- ³ Janowitz, H. D., and Grossman, M. I., *Am. J. Physiol.*, **159**, 143 (1949).
- ⁴ Pavlov, I. P., *The Work of the Digestive Glands* (translated by W. H. Thompson), second English edition, 53 (Charles Griffin, London, 1910).
- ⁵ Meyer, J. H., and Grossman, M. I., in *Gastrointestinal Hormones* (edit. by Demling, L.), 43 (Georg Thieme Verlag, Stuttgart, 1972).
- ⁶ Paré, W. P., *J. comp. physiol. Psychol.*, **80**, 150 (1972).
- ⁷ Gibbs, J., Young, R. C., and Smith, G. P., *Fed. Proc.*, **31**, 397 (1972).
- ⁸ Gibbs, J., Young, R. C., and Smith, G. P., *J. comp. physiol. Psychol.* (in the press).
- ⁹ Rubin, B., Engel, S. L., Drungis, A. M., Dzelzkalns, M., Grigas, E. O., Waugh, M. H., and Yiacas, E., *J. pharm. Sci.*, **58**, 955 (1969).
- ¹⁰ Johnson, L. R., Stening, G. F., and Grossman, M. I., *Gastroenterology*, **58**, 208 (1970).
- ¹¹ Ondetti, M. A., Rubin, B., Engel, S. L., Pluščec, J., and Sheehan, J. T., *Am. J. dig. Dis.*, **15**, 149 (1970).
- ¹² Dockray, G. J., *J. Physiol. Lond.*, **225**, 679 (1972).
- ¹³ Chey, W. Y., Sivasomboon, B., and Hendricks, J., *Am. J. Physiol.*, **224**, 852 (1973).

Methylguanidine, a Naturally Occurring Compound showing Mutagenicity after Nitrosation in Gastric Juice

Druckrey¹ and Sander² suggested that N-nitroso compounds may be formed intragastrically by reaction of nitrites with secondary amines or alkylureas present in ingested foods and that these natural compounds may hence be significantly carcinogenic in man. Sugimura *et al.*^{3–5} found that the muta-

gen, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)^{6,7} induced a high frequency of tumours in the glandular region of the rodent and dog stomach on oral application. MNNG is synthesized by nitrosation of N-methyl-N'-nitroguanidine (MNG) and the reaction occurs not only in strongly acidic conditions⁸ but also in human gastric juice (to be published). Therefore 'spontaneous' human gastric cancers may be at least partly caused by MNNG-like compounds formed after intragastric nitrosation of chemicals structurally related to MNG. We examined the mutagenicity of naturally occurring guanidines after treatment with nitrite in real and in simulated human gastric juice and found that methylguanidine, a compound present in several foods, is converted into a potent mutagen after nitrosation in gastric juice.

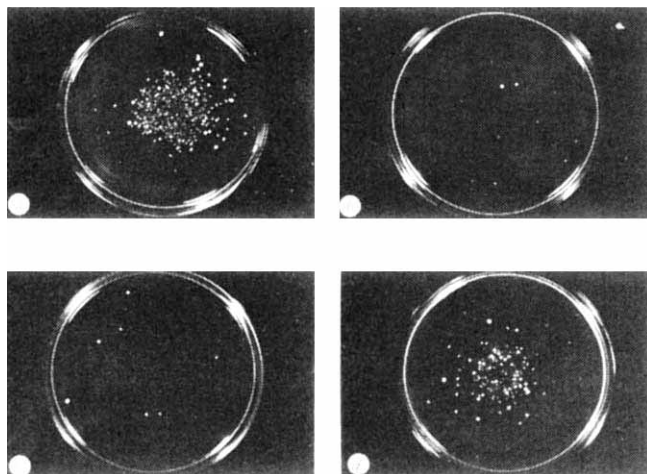


Fig. 1 Photograph of spot tests demonstrating mutagenicity of nitrosated methylguanidine. Approximately 1×10^8 *Salmonella* histidine-requiring mutants (TA1535) were plated on minimal agar (30 ml in 90 mm Petri plate) with 0.1 ml of nutrient broth, 0.1 μmol of histidine and 0.1 μmol of biotin (Ames⁹). The reaction mixture A contained 4 ml of simulated gastric juice which was composed of 200 mg sodium chloride, 320 mg pepsin and 2.4 ml of 10% hydrochloric acid in 100 ml (Japan Pharmacopoeia VII); 0.2 mmol of methylguanidine hydrochloride (Nakarai Chemical Co., Kyoto); and 0.22 mmol of sodium nitrite in a total volume of 5 ml. After incubation for 2 h at 37° C, the reaction mixture was neutralised with sodium carbonate, adjusted to 6 ml with phosphate buffer (pH 7.2), sterilised by Millipore (HA-type) filtration and spotted on a lawn of TA1535. The reaction mixture B was the same as A except that human gastric juice was used instead of simulated gastric juice. After incubation and neutralisation, 16 mg of Pronase-P (Kaken Chemical Co., Tokyo) was added to the mixture to make it filtrable. After incubation for a further 30 min, reaction mixture B was treated as above. The plates were incubated for 2 d at 37° C. a, Reaction mixture A; b, control mixture A, lacking methylguanidine; c, control mixture A, lacking sodium nitrite; d, reaction mixture B, containing human gastric juice, pH 1.2.

We investigated the following naturally occurring guanidines: methylguanidine (MG), agmatine, γ -guanidinobutylic acid, L-arginine, α -N-acetyl-L-arginine, α -N-acetyl- β -hydroxy-L-arginine, canavanine, D-octopine, phosphoarginine, phosphocreatine, creatine, creatinine, glycocyamine and taurocyamine. The bacteria used for the mutagenicity assay were deep rough derivatives of histidine-requiring strains of *Salmonella typhimurium* carrying the *gal-bio-uvrB* deletion: TA1535(hisG46) is used to identify mutagens causing base-pair substitutions, and TA1536(hisC207), TA1537(hisC3076) and TA1538(hisD3052) are used to identify mutagens causing various types of frameshift mutations. The nitrosation reaction mixture A contained a guanidine derivative to be tested, sodium nitrite and hydrochloric acid (simulated gastric juice) in a molar ratio of 1:1:1.3. The mutagenicity assay was carried out by spotting samples from the reaction mixture

on to lawns of tester strains of bacteria which had been plated on semi-enriched minimal agar 1 h in advance. After incubation at 37° C for about 2 d, revertants were observed.

As shown in Fig. 1a, a large number of colonies of strain TA1535 appeared after spotting with undiluted reaction mixture A. Ten colonies randomly picked from the plate were all his⁺ and as sensitive to ultraviolet radiation as TA1535. Controls lacking either MG (Fig. 1b) or nitrite (Fig. 1c) caused an average of only six or seven colonies to appear. Reaction mixture A caused no increase in the number of colonies of the other strains of bacteria, TA1536, TA1537 and TA1538. Therefore the colonies shown in Fig. 1a are almost certainly his⁺ revertants of TA1535 which appear because a mutagen causing base-pair substitutions is produced in the reaction mixture. Nitrosated MG was found to be mutagenic at up to sixteen-fold dilution of the original reaction mixture. All the other guanidines tested failed to show the mutagenicity detectable by this spot test.

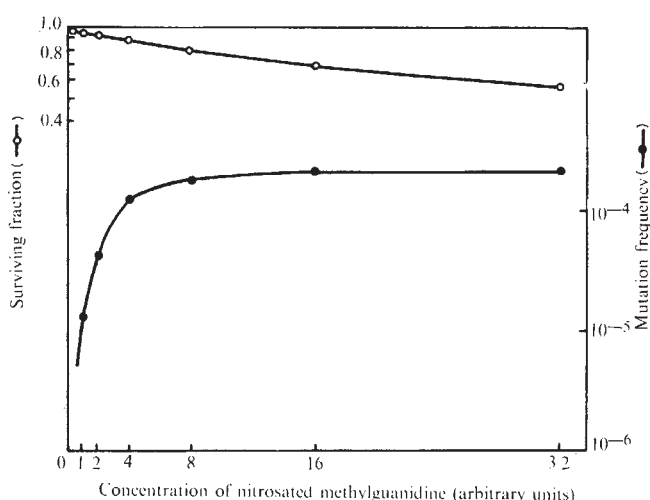


Fig. 2 An overnight culture of TA1535 cells in nutrient broth was washed once and resuspended in M9 buffer at a concentration of 4×10^8 cells per ml. A 0.5 ml aliquot of the cell suspension was added to an equal volume of the reaction mixture A at a given concentration and incubated at 37° C for 30 min. The cells were washed once at 0° C and resuspended in 5 ml of M9 buffer. Revertants and survivals were scored after plating a 0.1 ml aliquot of the cell suspension undiluted or in appropriate dilution on minimal agar together with the same amount of nutrient broth, histidine and biotin as described in the legend to Fig. 1. The mutation frequency is the ratio of revertants to surviving colonies. Concentration of nitrosated methylguanidine is one unit when reaction mixture is diluted 256-fold.

The mutagenicity of the reaction mixture A was further studied quantitatively. As shown in Fig. 2, the mutation frequency increased markedly and reached a plateau with increased concentration of the reaction mixture, while the extent of survival decreased slightly. The maximal mutation frequency was about 2.2×10^{-4} , more than 1,000 times greater than the spontaneous mutation frequency of TA1535 cells (1.6×10^{-7}).

The mutagenicity of MG nitrosated in human gastric juice was studied by the spot test. Fresh samples of gastric juice with pH 1.2, 1.3, 1.4 and 3.4 respectively, were obtained from four patients with gastric ulcers and used in reaction mixture B. Many revertant colonies of strain TA1535 appeared after spotting with reaction mixture containing human gastric juice, with pH 1.2 (Fig. 1d), 1.3 or 1.4, but not with the reaction mixture containing gastric juice of pH 3.4. MG nitrosated in human gastric juice with pH 1.2 was mutagenic at up to sixteen-fold dilution of the original reaction mixture, like MG nitrosated in simulated gastric juice.

Our finding that the reaction mixture containing gastric juice at pH 3.4 was not mutagenic is in accordance with a report¹² that the intragastric nitrosation of secondary amines is sensitive to the pH of gastric juice.

Mirvish^{13,14} has studied the nitrosation of MG kinetically, and found that the final product of the reaction was methyl-nitroso-urea, but that methyl-nitrosocyanamide was transiently formed during the reaction. He suggested that the *in vivo* nitroso-urea, but that methyl-nitrosocyanamide was transiently of human gastric cancer, since the rate of *in vitro* nitrosation of MG to give methyl-nitroso-urea is slow. Our previous studies on the powerful mutagenicity of nitrosated benzoyl-L-arginineamide (BAA)¹⁵ and acetyl-L-arginineamide (AAA) (unpublished) suggest, however, that it is the methyl-nitrosocyanamide formed during the nitrosation of MG that is the biologically important compound, since the active principles of nitrosated BAA and AAA were identified as 4-benzoylamido-4-carboxamido-*n*-(N-nitroso)-butylcyanamide and 4-acetylamido-4-carboxamido-*n*-(N-nitroso)-butylcyanamide.

MG is structurally quite similar to MNG, and is found in fresh beef^{16,17}, ray¹⁸, cod¹⁷, sardines¹⁹, and especially in sharks¹⁸, at quite high concentrations (1,900 mg kg⁻¹ fresh weight). It therefore seems possible that the nitrosated MG is one of the causes of "spontaneous" human gastric cancers. We are now investigating the identity of the mutagenic principle produced in the nitrosation of MG, and administering MG and nitrite orally to animals in order to determine whether gastric cancers arise.

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- Druckrey, H., Steinhof, D., Beuthner, H., Schneider, H., and Klarner, P., *Arzneimittel-Forsch.*, **13**, 320 (1963).
- Sander, J., *Arch. Hyg. Bakt.*, **151**, 22 (1967).
- Sugimura, T., and Fujimura, S., *Nature*, **216**, 943 (1967).
- Sugimura, T., Fujimura, S., and Baba, T., *Cancer Res.*, **30**, 455 (1970).
- Sugimura, T., Fujimura, S., Kosuge, K., Baba, T., Saito, T., Nagao, M., Hosoi, H., Shimamoto, Y., and Yokoshima, T., *Gann Monograph* **8**, 157 (1969).
- Mandell, J. D., and Greenberg, J. A., *Biochem. biophys. Res. Comm.*, **3**, 575 (1960).
- Adelberg, E. A., Mandel, M., and Ching Chen, G. C., *Biochem. biophys. Res. Comm.*, **18**, 788 (1965).
- McKay, A. F., and Wright, G. F., *J. Am. chem. Soc.*, **69**, 3028 (1947).
- Ames, B. N., in *Chemical Mutagens: Principles and Methods for their Detection* (edit. by Hollaender, A.), **1**, 267 (Plenum Press, N.Y., 1971).
- Ames, B. N., Sims, P., and Grover, P. S., *Science*, **176**, 47 (1972).
- Ames, B. N., Gurney, E. G., Miller, J. A., and Bartsch, H., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 3128 (1972).
- Sander, J., Schweinsberg, F., and Menz, H. P., *Hoppe-Seyler's Z. physiol. Chem.*, **349**, 1691 (1968).
- Mirvish, S. S., *J. natn. Cancer Inst.*, **46**, 1183 (1971).
- Mirvish, S. S., in *Topics in Chemical Carcinogenesis* (edit. by Nakahara, W., Takayama, S., Sugimura, T., and Odashima, S.), 279 (University of Tokyo Press, 1972).
- Endo, H., and Takahashi, K., *Biochem. biophys. Res. Comm.*, **52**, 254 (1973).
- Komarow, S. A., *Biochem. Z.*, **211**, 326 (1929).
- Kapeller-Adler, R., and Krael, J., *Biochem. Z.*, **221**, 437 (1930).
- Kapeller-Adler, R., and Krael, J., *Biochem. Z.*, **224**, 364 (1930).
- Sasaki, A., *Tohoku J. exp. Med.*, **34**, 561 (1938).

Intestinal Calcium Binding Protein in the Diabetic Rat

A SPECIFIC duodenal calcium binding protein (CaBP) has been implicated in calcium absorption in many animal species¹ and adaptation of duodenal adsorption to increased calcium intake is accompanied by its increase. Vitamin D deficiency depletes and repletion restores CaBP with corresponding changes in duodenal absorption. The exact role of CaBP in calcium absorption has, however, not yet been defined. We studied calcium absorption in the diabetic rat, in which model system previous studies have shown enhanced carbohydrate and amino acid absorption^{2,3}, and found decreased duodenal but normal ileal absorption¹. Since CaBP may be important in duodenal calcium absorption, we report here studies of CaBP in rats made diabetic with alloxan.

Table 1 Duodenal Calcium Binding Protein in Control and Diabetic Rats

Groups, <i>n</i> =5	C	D
Mucosa, total wet weight (g)	3.6	4.2
Homogenate, total protein (mg)	293	340
CaBP specific activity*		
Homogenate	1.2	0.6
'Bio-Gel' P-100†	18.1	1.2
'Bio-Gel' P-10, first	40.8	2.9
'Bio-Gel' P-10, second	59.0	3.7

Male albino rats weighing 180 to 200 g were divided into weight-matched groups. Controls (C) were injected intraperitoneally with 0.5 ml of distilled water; rats to be made diabetic (D) received 210 mg kg⁻¹ of a freshly prepared solution of alloxan in distilled water (100 mg ml⁻¹). On the fifth day after injection, groups of five control and five diabetic rats were anaesthetized with intraperitoneal 'Dial' with urethane and the 10 cm of small intestine just distal to the pylorus (duodenum) removed. After blotting and rinsing the lumen with cold 0.12 M sodium chloride, the segment was opened longitudinally, the mucosa scraped from the underlying tissue and pooled for each group, weighed and homogenized with an equal volume of 80 mM Tris buffer⁵. The homogenate was centrifuged at 40,000*g* for 20 min, the sediment resuspended, and the extraction procedure repeated twice. The three extracts were combined and analysed for total protein⁶ and CaBP activity⁵. CaBP activity was monitored by the 'Chelex' assay, which measures the competition for added radiocalcium between soluble CaBP in the supernatant and 'Chelex' (Bio-Rad Laboratory, Richmond, California), an insoluble calcium chelating resin easily separated from solution by centrifugation. Calcium binding activity is expressed as % ⁴⁵Ca retained in the supernatant fluid per mg protein and, therefore, tightly bound to soluble protein. A 13 ml sample of the crude supernatant fluid was applied to a 'Bio-gel' P-100 chromatographic column (2.5 × 120 cm) and eluted with Tris buffer by ascending flow at a rate of 0.5 ml min⁻¹. Fractions were collected in 5 ml aliquots. Protein concentration of each fraction was measured by absorbance at 280 nm (recovery 80 to 100%) and CaBP activity was determined as above. Fractions containing CaBP activity (75 ml) were combined and fractionated on a 'Bio-gel' P-10 column and protein and CaBP activity determined.

Duplicate experiments gave the same relative data.

* Specific activity is % ⁴⁵Ca in supernatant mg⁻¹ per ml protein.

† Specific activities of all gel filtration studies are from the fraction showing peak activity.

Although duodenal mucosal wet weight and total protein were slightly greater in the diabetics, CaBP activity was markedly decreased (Table 1). Fractionation of the control duodenal extracts using 'Bio-gel' P-100 revealed a sharp peak of CaBP activity (18.1) at approximately 2.7 void volumes. The elution pattern of both CaBP activity and protein was similar to that found previously in the normal duodenum using 'Sephadex' G-100⁵. Fractionation of the duodenal extracts from diabetic rats showed no peak of CaBP activity (1.2). Chromatography on 'Bio-gel' P-10 of the 'Bio-gel' P-100 fractions from controls and diabetics revealed protein peaks at the same sites in both groups. The controls continued to exhibit high CaBP activity (40.8) but the diabetics again showed no peak of activity (2.9).

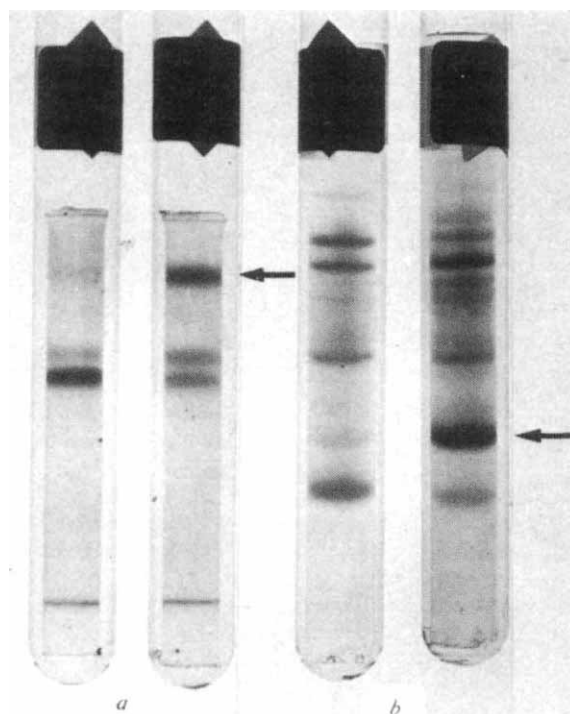


Fig. 1 'Bio-gel' P-10 fractions (Table 1, first) containing peak CaBP activity from controls and diabetics were concentrated on 'Diaflo' UM-2 membranes (Amicon Corporation, Lexington) and subjected to acrylamide gel electrophoresis in two systems, one a discontinuous basic system (a) using 7.5% acrylamide gels at pH 8.9 (ref. 9) and the other a continuous acid system (b) using 15% acrylamide gels at pH 3.2 (ref. 10). In the basic system, the control preparation exhibited a prominent slow moving band (arrow), shown to be CaBP by 'Chelex' assay, which was markedly reduced in the preparation from the diabetics. The prominent CaBP band in controls (arrow), again as determined by 'Chelex' assay of the gels, was the second most rapidly migrating band in the acid gel system. As in the basic system, the CaBP band was virtually absent in the preparation from the diabetics. All other protein zones from both gel systems were assayed by the 'Chelex' method and failed to show calcium binding activity.

Repeated 'Bio-gel' P-10 chromatography of fractions from controls and diabetics again demonstrated no CaBP activity in the diabetics (controls, 59.0; diabetics, 3.7), although the protein elution peaks continued to be present at the same sites in both groups. Chromatography enhanced CaBP specific activity forty-nine-fold in controls and six-fold in diabetics (Table 1). The initial specific activity in the diabetics was half that of controls, while final specific activity in the controls was sixteen times that in the diabetics.

We demonstrated previously that duodenal calcium absorption in the diabetic rat was less than half that in controls⁴. Here we demonstrate that CaBP was markedly reduced in the diabetic rat. Therefore, we have shown that a direct correlation between duodenal calcium absorption and CaBP activity exists in the rat in a model system completely independent of those usually used for modifying calcium transport such as low calcium diet and vitamin D depletion and repletion¹. Streptozotocin diabetes also produces decreased CaBP. Alloxan treated rats that do not develop diabetes have the same CaBP content as matched controls. Therefore, depression of CaBP in the alloxan diabetic rat seems to be the consequence of diabetes, rather than alloxan toxicity. In addition, effects in this system are rapid, requiring only 5 d to deplete the animal of calcium binding protein, in contrast to vitamin D depletion which requires 4 to 8 weeks. The diabetic rat also shows increased duodenal mucosal growth in spite of depression of calcium transport activity. This is in contrast

to the vitamin D deficient animal, where mucosal growth is depressed⁷. Thus, the diabetic rat provides a new model system for examining the role of CaBP in duodenal calcium transport and studying control mechanisms for intestinal transport, as the intestine of the diabetic rat shows both increased and decreased transport activity for carbohydrates and calcium, respectively. As a calcium binding protein has been isolated from human duodenum⁸, it is possible that a defect similar to that in the rat develops in poorly controlled patients with diabetes.

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- ¹ Wasserman, R. H., and Taylor, A. N., in *Mineral Metabolism, An Advanced Treatise* (edit. by Comar, C. L., and Bronner, F.), 3 (Academic Press, New York, 1969).
- ² Schedl, H. P., and Wilson, H. D., *Am. J. Physiol.*, **220**, 1739 (1971).
- ³ Lal, D., and Schedl, H. P., *Clin. Res.*, **20**, 733 (1972).
- ⁴ Schneider, L. E., and Schedl, H. P., *Am. J. Physiol.*, **223**, 1319 (1972).
- ⁵ Drescher, D., and DeLuca, H. F., *Biochemistry, N.Y.*, **10**, 2302 (1971).
- ⁶ Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. biol. Chem.*, **193**, 265 (1951).
- ⁷ Urban, E., and Schedl, H. P., *Experientia*, **25**, 1270 (1969).
- ⁸ Alpers, D. H., Lee, S. W., and Avioli, L. V., *Gastroenterology*, **62**, 559 (1972).
- ⁹ Davis, B. J., *Ann. N.Y. Acad. Sci.*, **121**, 404 (1964).
- ¹⁰ Panyim, S., and Chalkley, R., *Arch. Biochem. Biophys.*, **130**, 337 (1969).

Effect of Testosterone on Sperm Maturation *in vitro*

SPERMATOZOA mature while passing through the epididymis. In rabbit, fertilizing ability is acquired when spermatozoa pass through the corpus epididymidis^{1,2}. Spermatozoa retained by ligatures in the proximal corpus develop a normal fertilizing ability in 24 h in intact males^{3,4}, but not in castrated males⁵ or when the proximal corpus is maintained for 24 h *in vitro* in organ culture⁶. Spermatozoa from a ligated proximal corpus of a castrated male become fertile only when exogenous testosterone is given⁴. This indicates that rabbit sperm maturation is testosterone dependent. A technique for maintaining viable spermatozoa in epididymal segments in organ culture *in vitro* has been described⁶. Here we report the effect on the fertilizing ability of spermatozoa from the rabbit proximal corpus of adding testosterone to the culture medium.

The fertilizing ability of the spermatozoa from the control proximal corpus, the control distal corpus and the distal corpus maintained *in vitro* was determined in each male to ensure that (1) the male was fertile; (2) the spermatozoa from the proximal corpus were not already fertile (this occurs in approximately 13% of the males³; and (3) the conditions of the organ culture were sufficient to maintain the fertilizing ability of spermatozoa from the distal corpus. Of the sixty-nine males used, four had

infertile spermatozoa in all epididymal segments, eleven had spermatozoa already fertile in the proximal corpus prior to the organ culture *in vitro*, seven had infertile spermatozoa in the control distal corpus although those more distal were fertile, eighteen had no fertile spermatozoa in the distal corpus at the end of the culture period, although spermatozoa from the control distal corpus had a normal fertilizing ability. Results are given for all the fertile males (fifty-four) in which spermatozoa from the control contralateral proximal corpus were infertile (group 1) and for the twenty-nine of these in which the fertilizing ability of the spermatozoa from the distal corpus was optimally maintained during the culture (group 2). When the proximal corpus epididymidis of the males of group 1 was

Table 1 Fertilizing Ability of Rabbit Spermatozoa* from the Corpus Epididymidis Maintained in Organ Culture for 24 h

		Mean fertilization percentage $\pm$ sd†		
		Eagle's medium	Eagle's medium + testosterone (0.5 μ g ml ⁻¹)	Eagle's medium + testosterone (50 μ g ml ⁻¹)
Proximal corpus	Control	0† (0)§	0 (0)	0 (0)
	<i>in vitro</i>	0 (0)	13 $\pm$ 5 (20 $\pm$ 10)	11 $\pm$ 5 (18 $\pm$ 9)
		61 $\pm$ 10 (72 $\pm$ 9)	35 $\pm$ 6 (46 $\pm$ 9)	45 $\pm$ 7 (55 $\pm$ 7)
Distal corpus	Control	61 $\pm$ 10 (72 $\pm$ 9)	35 $\pm$ 6 (46 $\pm$ 9)	45 $\pm$ 7 (55 $\pm$ 7)
	<i>in vitro</i>	55 $\pm$ 11 (75 $\pm$ 8)	33 $\pm$ 7 (54 $\pm$ 12)	37 $\pm$ 9 (55 $\pm$ 12)

* Number of sperm inseminated: $1.2 \pm 0.04 \times 10^6$.

† Fertilization %: number ova fertilized/number ova recovered.

‡ Results calculated from all fertile males with infertile spermatozoa in the proximal corpus (fifty-four males).

§ Results calculated from all males with infertile spermatozoa in the proximal corpus and fertile spermatozoa in the distal corpus before and after culture *in vitro* (twenty-nine males).

|| Distal corpus cultured in Eagle's minimal medium without testosterone.

One testis was reached by means of an abdominal incision in the anaesthetized animal. The connective tissue and fat were peeled from the epididymis. The proximal and distal corpus were removed and each placed in 1 ml of a solution of 0.5% clostridium peptidase for 30 to 60 min at 31° C. After twice rinsing, the epididymal tubule was gently unravelled and placed unbroken on an agar strip. Trowell's⁷ organ culture technique as modified by Steinberger⁸ was used. Eagle's⁹ minimal medium with 15% foetal calf serum and 100 IU penicillin-streptomycin mixture was used. The gas phase was air containing 5% CO₂, and the cultures were incubated at 31° C and at a pH of 7.0 $\pm$ 0.02. A 2 mm segment from the epididymis just distal to the distal corpus was also treated in an identical manner. After 24 h the epididymal tubules in organ culture were removed, cut up in 1 ml Hank's solution and the debris removed. The three sperm suspensions were injected through both uterine horns in one or two does. The does were injected with an ovulatory dose of 50 IU of human chorionic gonadotrophin (HCG) at the time of insemination and killed 26 h afterwards. The oviducts were flushed and the recovered eggs examined for the presence of pronuclei or of cleavage, taken as evidence of fertilization. Nuclear details were clarified with acetocarmine staining after fixation in 1:3 acetic alcohol¹⁰. The donor male was then killed; the contralateral testis and epididymis were removed and the proximal and distal corpus were separated, cut up in 0.5 ml Hank's solution and the debris discarded. Sperm suspensions were prepared and similarly inseminated into one or two does. Seventy-nine males and 510 females were used, and 3,676 ova were examined. Eagle's minimal medium was used in thirteen experiments. In thirty-three experiments 0.5 μ g ml⁻¹ of testosterone (Sigma) dissolved in absolute ethanol was added to the culture medium used for the proximal corpus. In twenty-three experiments 50 μ g ml⁻¹ of testosterone was added to the medium used to culture the proximal corpus. In forty-three experiments 50 μ g ml⁻¹ of absolute alcohol was added to the Eagle's medium used to culture the epididymal segment distal to the corpus. In ten experiments the fertilizing ability of spermatozoa from the right or the left proximal corpus was determined and found to be comparable: in two males where spermatozoa from the right proximal corpus fertilized 68% of thirty-five ova, those from the left proximal corpus fertilized 81% of thirty-two ova. In eight experiments spermatozoa from the left side fertilized none of 116 ova, those from the right side fertilized three of ninety-six ova. In the latter case the three fertilized ova came from three different females.

placed in Eagle's medium, these spermatozoa fertilized 0/38 ova when recovered 24 h later. When $0.5 \mu\text{g ml}^{-1}$ or $50 \mu\text{g ml}^{-1}$ of testosterone was added to the culture medium, spermatozoa fertilized respectively 48/248 and 20/158 of the ova. Of the males of group 2 only, with Eagle's minimal medium alone, spermatozoa from the proximal corpus fertilized none of the twenty-eight ova but when $0.5 \mu\text{g ml}^{-1}$ or $50 \mu\text{g ml}^{-1}$ of testosterone was added to the medium, respectively, 39/110 and 20/85 ova were fertilized. In Table 1 results are expressed as the mean of fertilization percentages obtained for each individual male. In the sixty-four experiments where the fertilizing ability of the spermatozoa from the distal corpus was tested before and after the culture, the mean fertilization percentages were not significantly different (49 ± 4 control, 40 ± 9 *in vitro*). In the forty-three experiments comparing the fertilizing ability of the distal corpus spermatozoa after culture with Eagle's medium alone or to which the volume of ethanol used to dissolve testosterone was added, the fertilization percentages were not statistically different (41 ± 8 *in vitro*, 30 ± 6 *in vitro* + ethanol).

It seems that in our conditions the tissues remain sufficiently viable to maintain the fertilizing ability of spermatozoa already fertile: 70% of 114 experiments yielded positive results. Under the same conditions, addition of testosterone to the proximal corpus permitted the maturation of the contained spermatozoa in only 37% of thirty-eight experiments. Addition of testosterone to the medium ($0.5 \mu\text{g}$ or $50 \mu\text{g ml}^{-1}$) significantly increases the fertilizing ability of spermatozoa from the proximal corpus cultured *in vitro* without testosterone. This fertilization percentage, however, is significantly lower ($0.5 < P < 0.02$) than the one obtained after insemination of spermatozoa from the distal corpus maintained *in vitro*. This indicates our *in vitro* system is only partially successful in promoting sperm maturation, and we are investigating other factors affecting sperm maturation *in vitro*.

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¹ Bedford, J. M., *J. exp. Zool.*, **163**, 319 (1966).

² Orgebin-Crist, M. C., *Ann. Biol. Anim. Biochem. Biophys.*, **7**, 373 (1967).

³ Bedford, J. M., *J. exp. Zool.*, **166**, 271 (1967).

⁴ Orgebin-Crist, M. C., *Nature*, **216**, 816 (1967).

⁵ Orgebin-Crist, M. C., Dyson, A. L. M. B., and Davies, J., *Fécondité et Stérilité du Mâle-Acquisitions Récentes*, 227 (Masson, Paris, 1972).

⁶ Orgebin-Crist, M. C., and Tichenor, P. L., *Nature*, **239**, 227 (1972).

⁷ Trowell, O. A., *Expl Cell Res.*, **16**, 118 (1959).

⁸ Steinberger, A., Steinberger, E., and Perloff, W. H., *Expl Cell Res.*, **36**, 19 (1964).

⁹ Eagle, H., *Science*, **N.Y.**, **130**, 432 (1959).

¹⁰ Darlington, C. D., and La Cour, L. F., *The Handling of Chromosomes* (fourth ed.) (Allen and Unwin, London, 1962).

Blood Group A Antigens on Human Trophoblast Cells

THERE is still no adequate explanation for the fact that the placenta, which is foetal in origin and therefore allogeneic in the mother, is not rejected during pregnancy. A question of fundamental importance is whether human trophoblast cells possess HL-A or ABO antigens. Evidence so far is

conflicting¹⁻⁴. We present evidence which suggests that blood group A antigens may be present on human trophoblast cells, but at low surface density.

Foetuses 12-13 weeks old and placentae were obtained from therapeutic abortion cases. Their blood groups were ascertained by grouping the foetal heart blood and the group A specimens were used for the experiments. Villi were dissected off the placentae, minced with a scalpel, and trypsinized in a 0.1% solution of trypsin (Difco-Bacto) using a magnetic stirrer. Foetal and adult skin were similarly treated. The first supernatant containing blood and debris was discarded but subsequent lots containing skin and trophoblast cells were retained, washed thrice, and resuspended in phosphate-buffered saline (PBS).

In the mixed agglutination experiment, the method described by Coombs *et al.*⁵ was used. The cell suspension was incubated with a heat-inactivated immune anti-A serum (143490) for 1 h at room temperature. The cells were washed twice and resuspended in 0.2% bovine serum albumin (BSA). One drop of the suspension was transferred to each of two precipitin tubes and one drop of a 0.5% suspension of group A or group O human red cells was added. After mixing and centrifugation for 2 min at 1,500 r.p.m., the suspension was examined by phase contrast microscopy for specific linking of red cells to trophoblast and skin cells. In addition, some trophoblast cells were incubated with neuraminidase (Koch-Light) for 30 min at 37° C in a concentration of 100 IU of neuraminidase per 10⁶ cells before the mixed agglutination experiment.

Table 1 Mixed Erythrocyte-Cell Agglutination

Cells	Cells treated with anti-A serum, washed, and added to red cell suspensions of group:	
	A	O
Adult skin (AS1)	+	-
Foetal skin (S42)	+	-
Foetal skin (S43)	+	-
Trophoblast (T30)	-	-
Trophoblast (T36)	-	-
Trophoblast (T37)	-	-
Trophoblast (T42)	-	-
Trophoblast (T43)	-	-
Trophoblast (T42) treated with neuraminidase	-	-
Trophoblast (T43) treated with neuraminidase	-	-

In the mixed antiglobulin experiment, the method described by Sell *et al.*⁶ was used. Indicator cells were prepared by treating a 2% suspension of group A red cells with a non-agglutinating dose of immune anti-A serum diluted 1 in 40. After incubation for 1 h at room temperature, the red cells were washed twice and resuspended as a 0.5% solution in 0.2% BSA. Two drops of trophoblast cell suspension were incubated with two drops of immune anti-A serum (143490) for 1 h at room temperature, washed twice and resuspended in 0.2% BSA. These cells were then treated with an equal volume of a rabbit anti-human globulin (RY6) diluted 1 in 20, left for 30 min, washed twice and resuspended in 0.2% BSA. Two drops of sensitized indicator red cells were then added, centrifuged for 2 min at 1,500 r.p.m. and resuspended gently with a pipette. The mixture was mounted on siliconed slides and examined using a phase contrast microscope. The experiment was repeated with the immune anti-A serum after it has been absorbed with human group A red cells.

For transmission electron microscopy, trophoblast and skin cell suspensions were incubated with immune anti-A serum (143490) for 30 min at room temperature. After

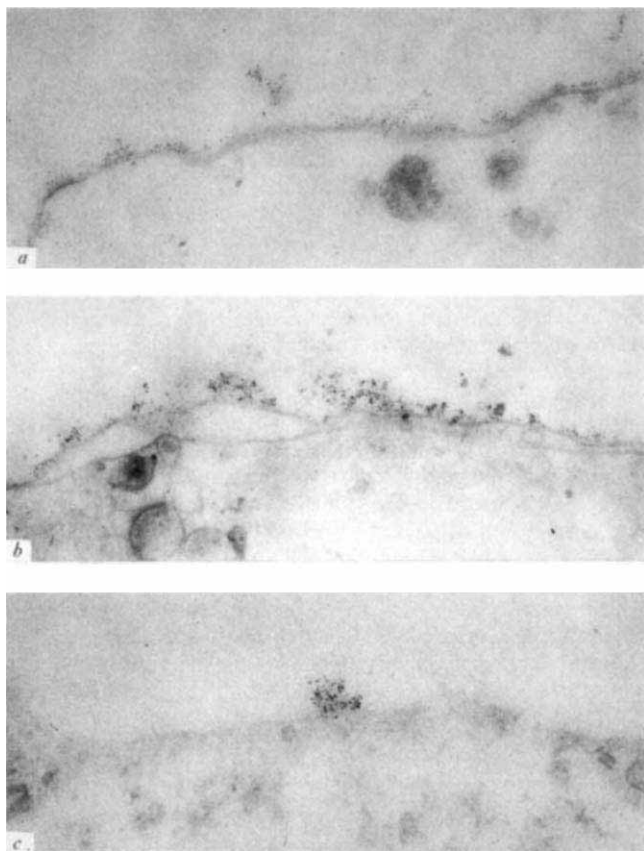


Fig. 1 *a*, Blood group A foetal skin cell treated with immune anti-A serum and ferritin-labelled anti-human IgG showing labelling over greater part of cell membrane. *b*, Blood group A adult skin cell similarly treated. *c*, Blood group A trophoblast cell treated with immune anti-A serum and ferritin-labelled anti-human IgG showing only discrete areas of labelling.

washing twice, the cells were incubated with ferritin-labelled sheep anti-human IgG (Wellcome) diluted 1 in 5 for 30 min at room temperature and then fixed in 2% osmium tetroxide containing 50% Michaelis buffer at pH 7.5. The cells were then washed with the buffer and with distilled water and embedded in 1 ml of 2% agar previously heated to 45° C. The agar block containing the cells was cut to the required size, dehydrated in alcohol and propylene oxide and embedded in 'Araldite'.

Adult (one sample) and foetal (two samples) skin cells showed positive mixed agglutination with group A red cells, but seven samples of trophoblast cells including two treated with neuraminidase were negative (Table 1).

In the experiment with antiglobulin, trophoblast cells reacted with unabsorbed anti-A serum, antiglobulin and group A red cells, but the reaction became negative when the anti-A serum was absorbed with group A red cells.

The electron micrographs showed ferritin labelling over the greater part of the cell membrane in foetal skin and adult skin cells (Fig. 1*a* and *b*) but, in contrast, only discrete, widely separated clumps were seen on trophoblast cells (Fig. 1*c*). The anti-A serum used has been tested against a panel of lymphocytes and no HL-A antibodies were demonstrable. Furthermore, the trophoblast and foetal skin cells were from the same conceptus so they would be expected to possess similar antigens. Controls in which the ferritin-labelled anti-human IgG was incubated with the cells without prior sensitization with anti-A serum were all negative.

It is unlikely that our results are due to masking of antigens on trophoblast cells by sialomucin as is sometimes suggested^{7,8} because prior treatment of the cells by neuraminidase made no detectable difference. A possible

interpretation of our results is that antigens on the trophoblast cell membrane are either less accessible or so sparse that insufficient antibody was taken up to form stable bridges directly with the indicator cells⁹ until another link was introduced in the form of an antiglobulin.

Our electron micrographs of immuno-ferritin-labelled sections offered additional evidence that blood group A antigens may be less dense on trophoblast cells.

The phenomenon of immunological enhancement has been cited as a possible mechanism by which the allogeneic placenta avoids maternal rejection¹⁰. Antigenic density of the target cells may be an important factor because fixation of antibody on to surface antigens which are sparsely distributed may lead to an enhancing effect¹¹.

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¹ Theide, H. A., Choate, J. W., Gardner, H. H., and Santay, H., *J. exp. Med.*, **121**, 1039 (1965).

² Gross, S. J., *Am. J. Obstet. Gynec.*, **95**, 1149 (1966).

³ Seigler, H. L., and Metzgar, R. S., *Transplantation*, **9**, 478 (1970).

⁴ Loke, Y. W., Joysey, V. C., and Borland, R., *Nature*, **232**, 403 (1971).

⁵ Coombs, R. R. A., Bedford, D., and Rouillard, L. M., *Lancet*, **i**, 461 (1956).

⁶ Sell, K. W., Mori, W., Rack, J. H., Gurner, B. W., and Coombs, R. R. A., *Br. J. exp. Path.*, **50**, 413 (1969).

⁷ Kirby, D. R. S., Billington, W. D., Bradbury, S., and Goldstein, D. J., *Nature*, **204**, 548 (1964).

⁸ Currie, G. A., Van Doorninck, W., and Bagshawe, K. D., *Nature*, **219**, 191 (1968).

⁹ Coombs, R. R. A., and Franks, D., in *Progress in Allergy* (edit. by Kallos, P., and Waksman, B. H.), **13**, 174 (Karger, Basel, 1969).

¹⁰ Anderson, J. M., *Nature's Transplant* (Butterworth, London, 1972).

¹¹ Linscott, W. D., *Nature*, **228**, 824 (1970).

Prolactin Secretion is Specifically Inhibited by Nickel

PROLACTIN (PRL) secretion from the mammalian anterior pituitary gland is apparently chiefly under hypothalamic tonic inhibition in contrast to the primarily stimulatory influence of the brain on the other trophic hormones¹. Extracts of mammalian hypothalamus inhibit the release of PRL *in vitro* and *in vivo*¹. We report here that PRL-inhibiting-factor (PIF) activity in certain fractions derived from acetic acid extracts of bovine hypothalamus was due to nickel ion (Ni²⁺) in the extracts. Commercial nickel salts and purified PIF from bovine brain specifically inhibit release of PRL *in vivo* in the rat and *in vitro* from bovine pituitary, (a) in basal conditions, (b) in the presence of copper ion which markedly enhances release of all trophic hormones, and (c) following brief exposure of the pituitary tissue to cold, another effective, non-specific stimulant of hormone release.

During the purification of PIF, attempts were made to elucidate the nature of the active substance. Hydrolysis of purified material of certain chromatographic fractions with HCl or digestion with sulphuric acid failed to abolish the inhibitory action on PRL release *in vitro*. This suggested that an organic component in those fractions was not necessary for the PIF activity. Analysis of metal

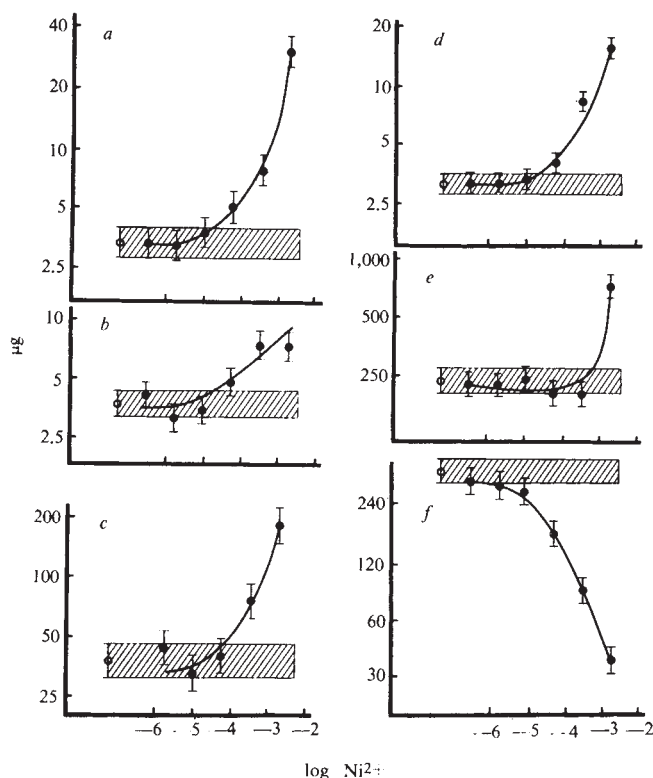


Fig. 1 Dose-response curve of nickel ion on hormone release *in vitro*. Bovine pituitary glands from animals of unspecified age and sex were obtained at a slaughterhouse 30 to 60 min after death. They were maintained at 25° C at all times before incubation at 37° C. For each experiment, ten to fifteen anterior lobes were sliced with a razor blade and diced into 1 mm cubes with a mechanical chopper. Homogeneous 300 mg portions of the diced tissue were added to 25 ml Erlenmeyer flasks containing 5 ml Krebs-Ringer-bicarbonate (with 200 mg % glucose), pH 7.4, and incubations carried out in a Dubnoff shaker at 37° C under 95% O₂/5% CO₂. The tissue portions were individually preincubated for 1 h with the medium renewed every 20 min. The preincubation medium was discarded, 5 ml fresh medium added, test substances added in 0.1 ml of buffer, and flasks were incubated for 2 h. Each experiment consisted of up to twelve treatments (several test substances at various concentrations and a control), each triplicated. The different treatments were assigned to the flasks in a randomized block pattern, each block consisting of twelve tissue aliquots which were weighed and added to the flasks consecutively over a short interval of time. The incubation medium was decanted, centrifuged to remove particulate material, and frozen. The medium was assayed for the bovine hormones by radioimmunoassay. The solid phase procedure² was used for PRL, growth hormone (GH), thyroid stimulating hormone (TSH), luteinizing hormone (LH) and follicle stimulating hormone (FSH) and the charcoal-dextran method for adrenocorticotrophic hormone³ (ACTH). The values for hormone levels in the media were log-transformed to eliminate variance heterogeneity. Subsequent statistical treatment consisted of a randomized block analysis of variance, which incorporated Tukey's test for non-additivity as an outlier screen⁴. The treatment means were compared with the control by Duncan's new multiple-range test⁴. We have previously described details of the *in vitro* incubation system and of statistical and technical aspects of the radioimmunoassays⁵⁻⁷. Each point represents three flasks, all five hormones being determined in the medium from each flask. Mean and s.e. are shown. a, TSH; b, LH; c, FSH; d, ACTH; e, GH; f, PRL.

metal ions, for example, copper and nickel, and which ordinarily prevent these metals from exerting their respective releasing or inhibiting actions *in vitro*. (Details of this work will be given elsewhere.)

Commercial nickel chloride specifically inhibited the basal release of PRL *in vitro* (Fig. 1). As the concentration of the metal was increased, PRL release was almost completely inhibited. Release of the other hormones was affected only at higher levels of nickel, where, in contrast to that of PRL, it was enhanced. Control experiments showed that nickel does not interfere with the radioimmunoassay for PRL.

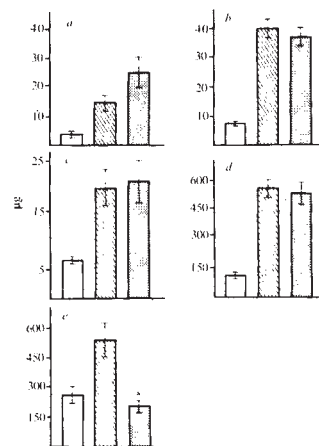


Fig. 2 Effect of nickel ion on the enhancement by copper ion of hormone release. □, Control; ▨, copper, 1 µg ml⁻¹; ▩, copper, 1 µg ml⁻¹ plus Ni²⁺, 5 µg ml⁻¹. Mean and s.e. are shown. a, TSH; b, LH; c, FSH; d, ACTH; e, GH; f, PRL. * Significantly different from Cu²⁺ alone (*P* < 0.01).

Cupric ion has been found to enhance release of all pituitary hormones *in vitro*⁹, as shown in Fig. 2. Nickel in a concentration of 5 µg ml⁻¹ inhibited the copper-induced release of PRL by 65%. No significant effects of nickel were observed on the copper-induced release of the other hormones.

We have previously observed that brief exposure of bovine anterior pituitary tissue to a temperature of 0° C results, on subsequent incubation at 37° C, in marked enhancement in release of all hormones¹⁰. Addition of nickel to the incubation medium just before cold exposure specifically blocks the enhanced release of PRL (Fig. 3).

In our *in vitro* system nickel ion specifically depresses the *in vitro* release of PRL, either in basal conditions or in circumstances in which release of all trophic hormones is markedly augmented. The cytoplasmic membrane of the lactotrope possesses unusual properties, with respect to responses *in vitro* to high concentrations of potassium¹¹, to cold¹⁰, and hypothalamic extracts¹. The unique response to nickel of the lactotrope, compared with other pituitary cells, further suggests the presence of an unusual cell membrane.

In the urethane-anaesthetized, chlorpromazine-treated rat, intravenous infusion of 100 µg of Ni²⁺ (as nickel chloride) results in a 40% decrease in serum PRL at 30 min with no significant change in levels of GH and TSH (Table 1). The larger dose of 200 µg Ni²⁺ (Table 1) promotes a comparable specific reduction in circulating PRL, but, in contrast to the smaller dose, maintains a significant inhibition even after 60 min. The exact site of action of Ni²⁺ on PRL secretion *in vivo* cannot be ascertained in our present study. Our *in vivo* results, however, are

content by atomic absorption showed that nickel was a prominent component of the active fractions. Certain fractions which enhanced release of hormones in general were found to be rich in copper: fractions containing high concentrations of both copper and nickel promoted release of all hormones except PRL the release of which was inhibited. Acid hydrolysis of certain of these PIF-containing fractions results in enhanced inhibition of PRL release with concomitant enhancement of release of the other hormones. Acid hydrolysis apparently destroys peptides which chelate

Table 1 Effect of Nickel Chloride, Purified PIF, and Hypothalamic Extract on Hormone Blood Levels in the Anaesthetized, Chlorpromazine-Treated Rat*

	PRL		GH		TSH	
	30 min	60 min	30 min	60 min	30 min	60 min
Saline	24 ± 7	52 ± 15	21 ± 4	33 ± 9	31 ± 2	29 ± 3
CPZ†—pretreated						
Saline	113 ± 8	175 ± 18	44 ± 8	17 ± 7	32 ± 2	26 ± 2
Ni ²⁺ (100 µg)	72 ± 6‡	134 ± 22§	41 ± 8	22 ± 5	32 ± 2	21 ± 2
Ni ²⁺ (200 µg)	66 ± 6‡	67 ± 19‡	37 ± 6	24 ± 6	33 ± 3	32 ± 2
PIF¶	35 ± 11‡		33 ± 8		31 ± 3	
Hypothalamic extract¶	68 ± 9‡		38 ± 3		38 ± 3	

Adult male Sprague-Dawley rats weighing 270–320 g were used for the *in vivo* studies. Groups of rats were anaesthetized with urethane (150 mg per 100 g body weight, intraperitoneally (i.p.); 30 min later, chlorpromazine (10 mg kg⁻¹, i.p.) in saline was injected, and after 60 min the test solutions were infused (i.v.). Thirty and 60 min after injection of the test substance, blood samples were taken, and the serum separated and stored at -20° C until assayed. Serum hormones were determined by solid phase radioimmunoassay; kits supplied by NIAMD-NIH were used for estimation of rat PRL and GH, and for bovine TSH, an assay similar to that reported by Reichlin *et al.*⁸ using a highly purified bovine TSH standard preparation of Pierce (20 IU mg⁻¹). We are as yet unable to assay LH, FSH, and ACTH in rat serum.

Means and s.e. are shown. Because of the deaths of a few animals there was an unequal number of rats per group, but an average of five per group.

* Values are in ng ml⁻¹.

† Chlorpromazine hydrochloride.

‡ Significantly different from CPZ + saline; *P* < 0.01.

§ Significantly different from CPZ + saline; *P* < 0.05.

¶ The injected aliquot contained < 0.3 µg Ni²⁺.

¶¶ Injected aliquot equivalent to five rat hypothalamic fragments and contained < 0.25 µg nickel. Extract supplied by NIAMD (Rat HE-RP-1).

compatible with a direct, specific inhibitory action of Ni²⁺ on the PRL cell, as demonstrated *in vitro*.

We have, thus far, been unable to produce a significant decrease in blood PRL with nickel in rats that were not pretreated with chlorpromazine. The elevated blood levels of PRL produced by the drug are consistently depressed by the metal. Perhaps in the untreated rat the PRL cell is already under maximum inhibition by the hypothalamic inhibitory hormone.

Nickel ion cannot account for all of the PIF activity of extracts of hypothalamic tissue. For example, purified preparations of PIF, in contrast to nickel, can inhibit the release of growth hormone, as well as that of PRL, *in vitro*¹². Nickel consistently stimulates release of growth hormone *in vitro*, but only at relatively high concentrations (Fig. 2). Also, in hypothalamic fractions or extract showing specific PIF activity, the concentration of nickel was too low to account for the observed inhibitory action (see Table 1).

Nickel, like other divalent cations, is to a large degree associated with other components isolated from brain tissue. These other substances apparently include amino acids and peptides. Because none of our PIF fractions has been found to be completely free of nickel, we suggest the

possibility that the active substance may be a complex containing this metal ion as an essential constituent.

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- Meites, J., in *Rec. progr. Horm. Res.*, **28**, 471 (1972).
- Catt, K. J., Niall, H. D., Treagear, G. W., and Burger, H. B., *J. clin. Endocr. Metab.*, **28**, 121 (1968).
- Donald, R. A., *J. Endocr.*, **41**, 499 (1968).
- Steel, R. G. D., and Torrie, J. H., *Principles and Procedures of Statistics* (McGraw-Hill, New York, 1960).
- LaBella, F. S., and Vivian, S., *Endocrinology*, **88**, 787 (1971).
- Vivian, S., and LaBella, F. S., *Mem. endocr. Soc.*, **19**, 203 (1971).
- Vivian, S., and LaBella, F. S., *J. clin. Endocr. Metab.*, **33**, 225 (1971).
- Reichlin, S., Martin, J. B., Boshans, R. L., Schalck, D. S., Pierce, J. G., and Bollinger, J., *Endocrinology*, **87**, 1022 (1970).
- LaBella, F. S., Dular, R., Vivian, S., and Queen, G., *Biochem. biophys. Res. Commun.*, **52**, 786 (1973).
- LaBella, F. S., Dular, R., and Vivian, S., *Endocrinology*, **92**, 1571 (1973).
- Parsons, J. A., *J. Physiol., Lond.*, **210**, 973 (1970).
- LaBella, F. S., Vivian, S., Dular, R., and Eddie, L., *Serono Foundation Conference on Hypothalamic Hypophysiotropic Hormones* (in the press).

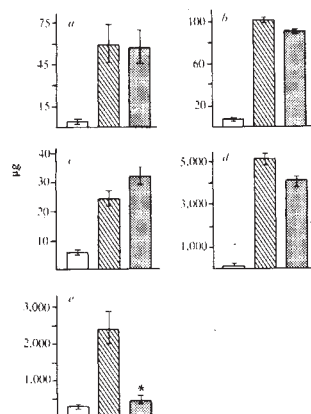


Fig. 3 Effect of nickel ion on the enhancement of hormone release by a 5 min exposure of the incubation flasks to 0° C. □, Control; ▨, cold exposure; ▤, cold exposure in the presence of Ni²⁺ (20 µg ml⁻¹). a, TSH; b, LH; c, ACTH; d, GH; e, PRL. * Significantly different from cold without Ni²⁺ (*P* < 0.01).

Social Control of Adult Size in Males of *Xiphophorus variatus*

As in other species of poeciliid fish, male *Xiphophorus variatus* (Pisces, Poeciliidae) virtually cease growth at maturity. The size of an adult male, then, is determined by its average growth rate before it reaches maturity and the age at which this happens. The latter factor is under social control in this species. When I

raised juvenile males in groups, they usually started to mature in sequence, largest first, and subsequently maturing males did not usually reach maturity until they were longer than all previously matured males. As a result, the juvenile size order was negatively correlated with adult size order. When the fish in a group were isolated from one another such patterns disappeared indicating that some degree of social interaction was necessary for their maintenance¹.

In addition to determining the size distribution within a group of adult males, these interactions also determine the distribution of the yellow or red pigmentation (YR coloration) that appears in the dorsal and caudal fins of some mature males. YR coloration develops only in the largest adult male in the group at any time starting at maturity and stopping when the male is exceeded in size by a newly matured male. Since males tend to mature at successively larger sizes, a number of males may develop some YR coloration with those having held the position of largest adult for the longest times the most vividly coloured ones in the group². Possession of YR coloration is of advantage in aggressive interactions with other males¹.

This system is an illustration of the phenomenon of social determination of phenotype, which includes social control of sex reversal in protogynous fishes^{3,4}. The phenomenon may be widespread among vertebrates, especially if such phenotypic characteristics as aggressive and sexual behaviour patterns are included.

I report here three experiments designed to isolate the social factors involved in the control of maturation in *X. variatus*. Pairs of males were maintained together in aquaria until maturity. The standard lengths of the fish as well as their stages of sexual development—the stages in the transformation of the anal fin into the gonopodium—were determined periodically. Juveniles came from broods in which a few males had already started to mature, indicating that the juveniles, although expected to remain juvenile for months if left with the others, could mature at any time¹. In the first experiment, pairs of juveniles from the same brood were placed together in plastic aquaria containing 3 l of water. In the second experiment, single juveniles were placed in identical aquaria and maintained in isolation until they started to mature. In some cases, on reaching the stage of sixteen segments in the third ray of the anal fin, a relatively early stage of maturation but one that indicates that the fish is committed to further maturation (my unpublished results), an adult male was placed in its aquarium and the pair maintained until the first fish had reached maturity. (During the maturation process, the third, fourth and fifth rays of the anal fin (3–5 complex) elongate by addition and lengthening of segments. The third ray contains between ten and thirteen segments in a juvenile and more than twenty-five in an adult. After the elongation is complete, the bony elements at the distal end of the 3–5 complex differentiate into what is called the tip complex.) In other cases the males were maintained in isolation. In the third experiment, single juveniles were placed in glass aquaria containing 8 l of water and an adult male was added on the same day or after delays of 2, 5, or 7 d.

The results of the first experiment are summarized in Table 1. There was considerable variation in the latency to initiation of maturation and the presence of one fish delayed the onset of maturation in the other. If this were not the case, the latencies for any given pair would as often be on the same side of the median value as on opposite sides. This, however, is not the case; seven times the latencies were on the same side of the median while seventeen times they were on different sides of the median ($P=0.032$). I conclude that the onset of maturation in the second fish to mature was delayed by the presence of the other fish.

Part of the variation in latency is also attributable to the difference in length between the two individuals in a pair. As Fig. 1 shows, the more similar two fish were in length, the longer it took for the first fish to start maturing. This significant relationship (Spearman's rank difference correlation coefficient =

0.658, $N=24$, $P<0.001$) suggests that a question—which fish is to start first—must be resolved and the more disparate the two individuals are in length, the more quickly the decision can be made. In six cases the smaller of the two started first after having grown more quickly in experimental conditions than did the other member of the pair. In eighteen cases the larger of the two had started first and in five of these the smaller of the two had grown faster. Table 2 shows that when two fish

Table 1 Lengths, Growth Rates and Latencies to the Start of Maturation for All Fish in Experiment 1

Pair	Length at T_0 (mm)	T_A	Growth rate during interval T_0-T_{A1} (% d)
A	30.1	17	0.086
	27.6	84	0.084
B	25.4	11	0.649
	21.9	46	0.550
C	25.6	7	0.432
	21.9	64	0.068
D	23.9	7	0.623
	22.6	59	0.313
E	23.0	47	0.576
	22.6	18	0.703
F	24.9	4	0.398
	24.6	46	0.602
G	23.5	126	0.388
	23.2	45	0.399
H	21.8	39	0.538
	21.2	50	0.373
I	29.6	10	0.248
	24.5	50	0.319
J	24.0	7	0.565
	22.2	28	0.621
K	27.2	17+*	0.164
	26.9	17	0.197
AA	30.0	36	0.168
	30.0	24	0.172
BB	27.0	36	0.301
	26.1	90	0.133
DD	29.9	90	0.372
	28.9	15	0.407
EE	29.3	30	0.256
	28.8	90+†	0.165
FF	26.8	13	0.511
	25.1	‡	0.181
HH	27.6	29	0.452
	27.2	48	0.383
II	26.9	62	0.646
	24.7	13	0.701
LL	23.6	41	0.448
	22.4	90+†	0.525
MM	24.0	48	0.461
	23.9	34	0.471
NN	23.7	29	0.447
	22.8	48	0.415
AAA	25.2	8	0.246
	23.3	70	0.054
FFF	24.6	16	0.224
	24.3	41	0.228
GGG	24.4	16	0.375
	23.7	16+*	0.335
HHH	25.6	16	0.169
	23.9	102+	0.129

Column 2 gives the lengths of the fish on the first day of the experiment (T_0). Column 3 gives the day on which each fish started to mature (T_A). Column 4 gives the growth rates during the interval T_0 to T_{A1} (the day on which the first fish in a pair started to mature). Growth rates were calculated from many measurements during the interval so the lengths of fish on day T_{A1} cannot be calculated exactly from the data but a close estimate can be made by raising the growth rate expressed as a proportion to the T_{A1} th power and multiplying by the length on T_0 . This suffices for all pairs save G, AA and MM and in these cases the exact lengths of the first and second fish on the appropriate T_{A1} were 27.2, 27.5, 31.2, 31.2, 28.0 and 28.0 mm respectively. The criterion for the commencement of maturation was fourteen segments in the third ray of the anal fin⁴.

* These fish were less developed than their companions and had begun development later.

† Some time after 90 d these fish had started to mature.

‡ This fish died before T_A .

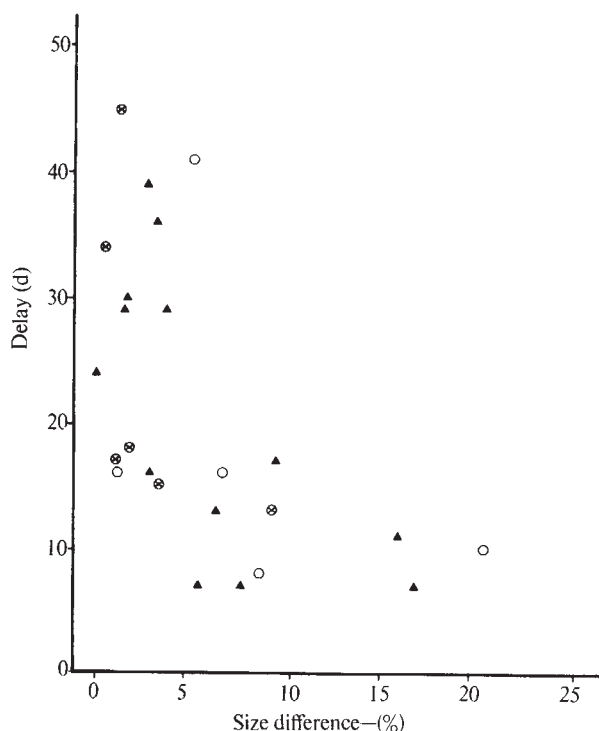


Fig. 1 The number of days before the first fish in a pair started to mature plotted against the difference in size between the two individuals expressed as a percentage of the size of the smaller. Circles denote pairs in which the smaller of the two grew faster during interval T_0 to T_{A1} (see Table 1) but started to mature second. Crossed circles denote pairs in which the smaller fish grew faster and matured first. Triangles denote pairs in which the larger fish grew faster and matured first.

were similar in size the deciding factor was relative growth rate and when they were dissimilar in size the deciding factor was relative size. (When the fish differed by less than 5% of the length of the smaller, twelve out of thirteen times (two-sided $P=0.0034$), the more rapidly growing individual started to mature first; but when the fish differed by more than 5%, ten out of eleven times (two-sided $P=0.012$) the larger individual started to mature first.) A single set of measurements will

suffice to determine relative sizes but a number of measurements made over time are necessary to determine relative growth rates. This may account for the longer latencies observed for similarly sized fish (Fig. 1) because in these cases the decision is made on the basis of relative growth rates and not relative sizes.

The results of the second experiment were clear cut. There was considerable variation in the number of days required for a transforming male to reach maturity when it was maintained in the presence of an adult male. There was less variability in this measure for the fish maintained in isolation. Figure 2 shows that the variability in the first case is related to the factor of relative sizes; when the sizes were similar the rate of transformation was retarded but when they were dissimilar there was no noticeable effect. This experiment shows that the rate of transformation during the latter stages of this process is under social control.

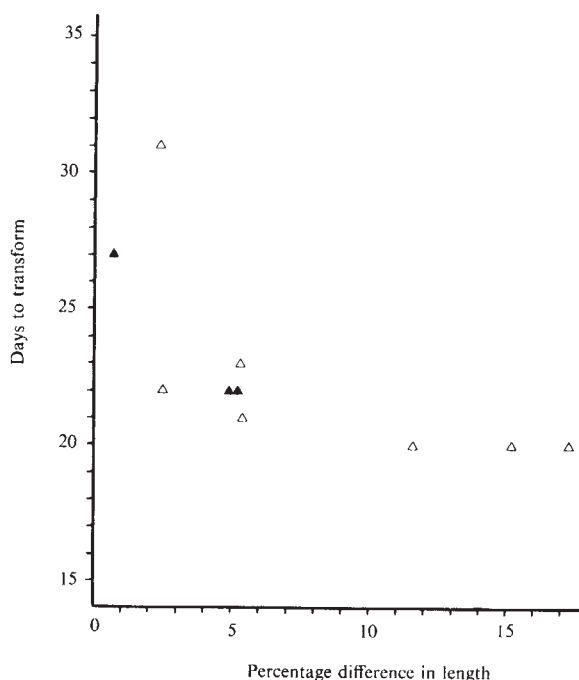


Fig. 2 Time necessary for a fish to develop from the stage of sixteen segments in the third ray of the anal fin to maturity plotted against the difference in size between the transforming fish and the adult placed with it. Average lengths during the transformation period, obtained by integrating the growth curves, were used. Open triangles denote pairs in which the adult was larger. Solid triangles denote pairs in which the adult was smaller. The five circles to the right of the graph give the number of days necessary for five unpaired fish to reach maturity under otherwise identical conditions. Spearman's correlation coefficient computed for all ten points: $r=0.89$, $P=0.00057$. Correlation coefficient computed for the seven open triangles: $r=0.93$, $P=0.0034$.

Table 2 Relationship between Maturation Order and Growth Rates

		The smaller of the two grows:		
		Faster	Slower	
A All pairs	The smaller of the two starts to mature			
	Later	5	13	18
	Earlier	6	0	6
		11	13	24
		Fisher's exact probability=0.0034		
B Less than 5%				
	Later	1	7	8
	Earlier	5	0	5
		6	7	13
		Fisher's exact probability=0.0047		
C More than 5%				
	Later	4	6	10
	Earlier	1	0	1
		5	6	11
		Fisher's exact probability=not significant		

A demonstrates that a relationship between relative growth rates within a pair and maturation order exists for experiment 1. B demonstrates that the relationship holds for pairs which initially differed by less than 5% of the length of the smaller. C shows that the relationship does not hold for pairs in which the initial difference in length was more than 5%. See text.

The results of the third experiment show that the day on which the adult fish was added to the aquarium was unimportant. The principal effect of adding the mature male was to delay considerably the onset of maturation in the juveniles. (The median latency for the four unpaired juveniles was 11 d while for the fourteen paired individuals it was 34 d. Wilcoxon's $T=12$, $P<0.01$.) Another important effect is seen in Fig. 3 which shows the rates of maturation for samples of unpaired and paired juveniles. One result of pairing in these circumstances was to decrease greatly the rate of transformation during the earlier stages of the process. Although the second fish to mature in the first experiment did not always exceed the first in size, there was a significant tendency for the

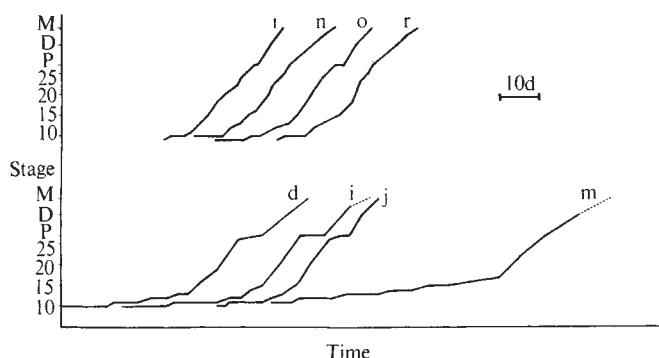


Fig. 3 Maturation curves for fish in experiment 3. Stage of sexual development is plotted against time for the four unpaired fish (above) and for the four fish paired on day 0 (below). The numbers on the ordinate are the number of divisions (one less than the number of segments) in the third anal fin ray. P, proximal hook stage of development of the tip complex; D, distal hook stage; M, mature. The two groups showed markedly different rates of maturation in the early stages of the process. Fish l, n, o, r, and d, i, j, m, took 6, 4, 9, 8, and 19, 23, 16, 33 + d respectively to proceed from stage 10 to stage 15. The medians for the two groups differ at the $P=0.014$ level. Fish i and m may have matured before they were checked finally so the last portions of the two curves are uncertain (dashed line).

second fish to be larger, which is in line with my earlier observations¹. (The median difference in adult length (length of second to mature minus length of first to mature) was 1.35 mm, $N=18$ pairs, Wilcoxon's signed rank test: $P=0.028$.)

Although from these and my earlier studies^{1,2} it would be logical to conclude that the "purpose" of these interactions is to allow each male in a population to be the largest adult male present for some time—a status attained by most males in laboratory groups of less than fifteen—this is not the case. I have sampled natural populations of these fish in Tamaulipas, Mexico, and found that they contain mature and maturing males of all sizes (my unpublished results). If the situation in the laboratory were an undistorted reflexion of that in nature, most of the maturing males in natural populations would be large relative to the sizes of the mature males. What then is the natural function of this complex adaptation? Presumably the adaptation allows a male to mature at any size that optimizes his fitness. A more specific answer requires new information, but one possibility is that the adaptation serves to fit maturing males into holes in the size distribution of adult males and that for some reason a male is in the best position when there are few similarly sized adult males. If females of a particular length respond more readily to the courtship of males of a particular length⁵ this would put similarly sized males in competition with one another and limit competition with dissimilarly sized males. In this light, it is notable that adult males that are similar in size tend to fight fiercely with one another while those of different sizes tend to ignore one another (ref. 1 and my unpublished results).

If the above interpretation is correct, the control over the time of initiation and the rate of maturation would serve as the coarse and fine adjustments respectively for easing a fish into a hole in the size distribution. For example, when a fish was a few millimetres short of such a gap in the distribution it would start to mature and by the time it had reached maturity and stopped growing it would have reached the proper size. If conditions had started to deteriorate so the fish could not have maintained its previous growth rate, it could still have matured at the desired size by retarding its rate of maturation, and thereby prolonging its period of growth. There is some evidence that this happens in laboratory groups. In my earlier study¹ I had noted that in all groups examined there was a positive correlation between a fish's length increase during transformation and the length increase that would have been necessary to attain the status of the largest adult present. There was also a

correlation between the rate of transformation and the latter factor. That is, the more the fish would have had to have grown to have matured as largest adult present, the more slowly it matured. In the laboratory where conditions for growth are not variable and probably nearly optimal, the most obvious gap in the size distribution is at the top. In natural populations where conditions can vary from optimal to lethal, fish might often opt to mature within the distribution. An analysis of the size distributions of mature and maturing males in natural populations is in progress.

In summary, the time of initiation of maturation in *X. variatus* is under social control, as is the rate of maturation during the very late and very early stages of the process. Only one other male need be present for this to occur. Larger or more rapidly growing juveniles or larger adults delay the initiation of the process; the rate of the process can be depressed by exposing a maturing male to a mature male. This effect is more pronounced when the two individuals are similar in length.

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¹ Borowsky, R. L., thesis, Yale Univ. (1969).

² Borowsky, R. L., *Physiol. Zool.*, **46**, 22 (1973).

³ Fishelson, L., *Nature*, **227**, 90 (1970).

⁴ Robertson, D. R., *Science*, **N.Y.**, **177**, 1007 (1972).

⁵ Barlow, G. W., *Z. Tierpsychol.*, **27**, 779 (1970).

Tibetan Species of Dung Beetle from Late Pleistocene Deposits in England

A LARGE and distinctive species of the dung beetle *Aphodius* that is clearly not a member of the present day European fauna has frequently been found in deposits in England that date from the middle of the Last Glaciation^{1,2}. Although at times very abundant (from one peaty lens in a gravel pit near Dorchester on Thames, fossils of at least 150 individuals were obtained) the identity of this species has up to now remained a mystery. The fact that the skeletal elements were to a large extent disarticulated made the use of keys to identification impossible and, with more than five hundred described species of *Aphodius* in the Palaearctic region alone, it would have been imprudent to describe the fossils as a new, possibly extinct, species. Thus in accounts of fossil assemblages of Coleoptera, this species has been given the non-committal designation of *Aphodius* sp. A of Upton Warren after the site in Worcestershire¹ where it was first found. The solution to the identity of this species was both unexpected and dramatic.

The only practical method of identifying fossils such as these is by a systematic search of comprehensive collections of modern Coleoptera. During one such search of uncatalogued specimens of *Aphodius* in the collections of the British Museum of Natural History, several unnamed specimens were found that matched precisely the problematical fossil species. These specimens were from the Hingston collection and had been obtained at high altitudes on the north slopes of the Himalayas during the 1924 British Everest Expedition. By reference to Balthasar's key³, these specimens were determined as *Aphodius holdereri* Reitter and confirmed by direct comparison with the holotype of this species loaned by the Natural History Museum, Budapest.

With the exception of the antennae and tarsal segments, fossils of all elements of the exoskeleton are available for

comparison with their modern counterparts. Figures 1 and 2 show a selection of these fossil fragments and present day *Aphodius holdereri* with which they can be compared. The precise correspondence between the two is unequivocal. Furthermore, closely related species such as *Aphodius semenovi* Reitter and *Aphodius hydrochoeris* Fabricius can be excluded as possible candidates because they differ from the fossils in numerous details of shape, sculpture, size and colour. There can thus be no reason to doubt that *Aphodius* sp. A of Upton Warren is in fact *Aphodius holdereri*.

This species is today restricted to the high Tibetan plateau where its range extends from the region of lake Koko Nor (now Ch'ing Hai) in the north to the north slopes of the Himalayas in the south. As far as I am aware there are no records from western Tibet. The localities from which it has been obtained range in altitude from 3,000 m to 5,000 m.

Fossils of *Aphodius holdereri* are widespread throughout the English Midlands. They seem to be confined to deposits that date from the middle phase of the Last Glaciation; a period which, fortunately, falls within the range of ^{14}C dating techniques. The oldest of these deposits was at Upton Warren, Worcestershire, from which a date of 42,000 BP (GRO 1245)¹ was obtained, and the youngest was at Thrapston, Northamptonshire, which gave a date of 26,000 BP (Birm 113)⁴. Out of a total of seventeen sites that produced insect assemblages dating from this period, fourteen yielded *Aphodius holdereri*. The species seems to have been most abundant at the very beginning of this period when it was our commonest large *Aphodius*, but after this its numbers gradually declined although it must have remained an important member of the British fauna throughout.

The occurrence of this Tibetan species in such relatively recent deposits in Britain has important zoogeographical implications. Not only does it illustrate the great changes of range that insect species have undergone in response to Pleistocene glaciations but it also points out the danger of trying to interpret faunal histories on the basis of present day distributions alone. In the absence of a fossil record, *Aphodius holdereri* might well have been interpreted as a typical Tibetan endemic species carrying the implication that it was "native and originating" there. Clearly the present day restriction of the range of such a species is more likely to be a reflection of the limited availability of acceptable habitats.

The past occurrence in lowland Britain of a species that is today confined entirely to altitudes greater than 3,000 m demands a reconsideration of the environmental factors responsible for this type of restriction. Mani⁵ considers that

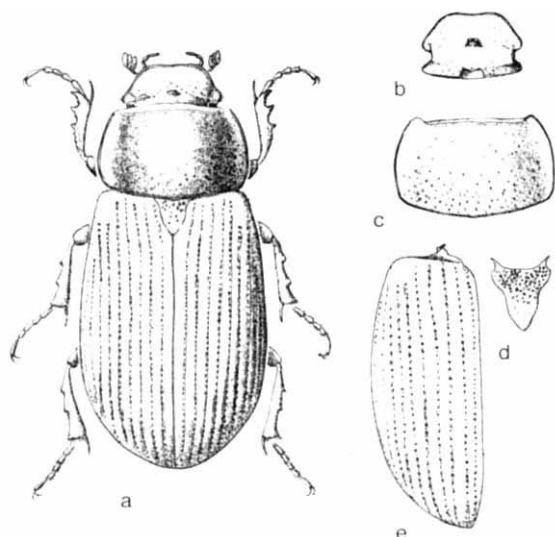


Fig. 1 Modern and fossil *Aphodius holdereri* Reitter. a, A modern ♂ specimen from the Himalayas. b, c, d and e, Fossils from Dorchester on Thames of head, pronotum, scutellum and left elytron. The elytron is distorted by flattening at the shoulder. All drawings are $\times 3$.

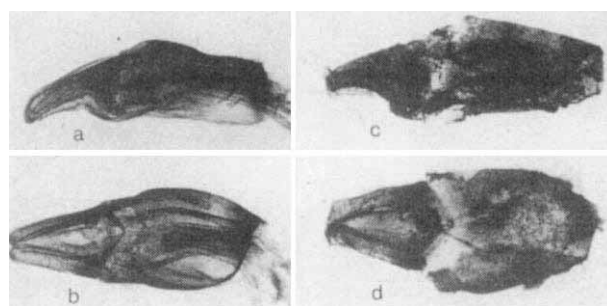


Fig. 2 Aedeagophores of modern and fossil *Aphodius holdereri* Reitter. a and b, Modern Himalayan specimens in lateral and dorsal view. c and d, Laterally and dorsoventrally compressed fossil specimens from Dorchester on Thames. The linear scale represents 1 mm.

the montane autochthonous insect species evolved in the mountains, presumably as adaptations to the peculiarities of the high altitude environment and *Aphodius holdereri*, fits well his definition of such hypsobiont species. Its occurrence in Britain during the Last Ice Age at altitudes very close to sea level suggests that there must have been environmental factors in common permitting the species to thrive in both situations. Unique characteristics of the high altitude environment such as low atmospheric pressures and the associated low oxygen tension and the extremely high insolation are excluded as environmental parameters determining the suitability of habitats in glacial Britain. Only similarities in the thermal environment are left as possible physical factors in common to the two situations. The restriction of certain insect species to high altitudes would thus seem to be determined almost entirely by the availability of suitable thermal conditions and the other peculiarities of the physical environment in these regions seem to be of minor significance.

I thank Dr Z. Kaszab for the loan of the type specimen of *Aphodius holdereri*, Dr R. B. Angus for his enthusiastic assistance and Rosemary Wise for the drawing of Fig. 1.

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¹ Coope, G. R., Shotton, F. W., and Strachan, I., *Phil. Trans. R. Soc.*, B, 244, 379 (1961).

² Coope, G. R., *Mitt. Limnol.*, 17, 173 (1969).

³ Balthasar, V., *Monographie der Scarabaeidae und Aphodiidae der palaearktischen und orientalischen Region* (Band 3, Prague, 1964).

⁴ Shotton, F. W., Blundell, D. J., and Williams, R. E. G., *Radio-carbon*, 12, 385 (1970).

⁵ Mani, M. S., *Ecology and Biogeography of High Altitude Insects* (The Hague, 1968).

Allergenic and Agricultural Implications of Airborne Ascospore Concentrations from a Fungus, *Didymella exitialis*

The occurrence of seasonal asthma resulting from fungal spores in late summer is well recognized. *Cladosporium* and *Alternaria* have been extensively studied as allergens¹ because of their worldwide distribution. Herxheimer *et al.*² used skin and bronchial testing when investigating allergic causes of recurrent summer asthma. They showed that certain types of basidiospores and one type of ascospore are antigenic in man. Seasonal symptoms occurring in late summer in Great Britain due to *Leptosphaeria* (*Phoma*) spp. have also been described³. Very large concentrations of ascospores, particularly in August, were noted by Hyde and Adams⁴. The clinical importance, especially of the large numbers of one-septate ascospores, has never been evaluated.

The air spora in the vicinity of a patient in Dorset whose August asthma was associated with proximity to large areas of barley was investigated⁵. A Burkard model of the Hirst⁶ spore trap was installed at 0.5 m above ground level in a garden near barley in the Blandford area. From July 10 to September 9, 1972, airborne particles in the 5 to 50 μm range were sampled continuously for subsequent microscopic examination which enabled the history of concentration changes to be reconstructed hour by hour. During the first 2 weeks sampling, it became clear that the local air spora was characterized by long periods with extremely dense concentrations of hyaline one-septate ascospores, often absent during the middle of the day, but reaching peak concentrations of 10^5 to 10^6 per m^3 of air at night (Fig. 1). These may be the "Group B" airborne ascospores from cereal crops depicted by Last⁷. They also resemble the ascospores found in dense concentrations in the air of wheat fields in Switzerland by Corbaz⁸, who identified them as *Didymella exitialis*.

None of the familiar fungi on barley produce ascospores of this type, yet such unusually dense concentrations in outdoor air indicate a very large source. Microscopic examination of random specimens of what seemed to be "normally withered" barley leaf fragments from the Blandford area and elsewhere revealed numerous perithecia, packed with asci and ascospores, immersed in the leaf tissue with only minute hyaline necks protruding through the epidermis (Fig. 2). These perithecia, which were found in every barley leaf examined in August and September 1972, numbered about 100 per cm^2 of leaf on average at Rothamsted and 200 to 300 at Blandford. Similar perithecia were found on wheat. Immature perithecia were abundant in barley flag leaves collected near Blandford on August 3.

Ascospores are discharged freely an hour or so after dried leaves are wetted in the laboratory. The continuous spore trap records show that in the field ascospores are liberated when leaves are wetted either by rain or dew. Cultures are easily obtained on the usual agar media.

Specimens on barley and cultures isolated from spores shed from barley leaves were examined. After comparison with type material the fungus was identified as *Didymella exitialis* (Moreau) Müller. Further isolations from the air over barley fields during summer are needed to establish whether other leaf fungi contribute substantially to the dense concentrations of one-septate ascospores observed.

Retrospective examination of the patient's diary showed that the symptoms developed in parallel with the ascospore concentrations, becoming worse during a period with concentrations of 1 to $2 \times 10^6 \text{ m}^{-3}$ during the first 2 weeks in August, and substantially disappearing when the spore concentrations declined as the barley harvest was completed by about August 26. Re-examination of trap samples from a similar study made near Cirencester in 1968, in the environment of a farmer

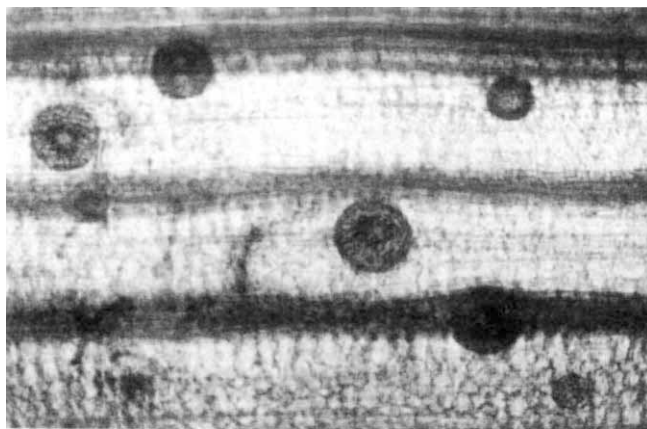


Fig. 2 Perithecia of *D. exitialis* in cleared barley leaf, collected near Blandford, Dorset, on August 3, 1972. Magnification: $\times 113.90$ approximately.

with "barley asthma", showed dense concentrations of similar ascospores.

As the correlations suggested a causal relationship, clinical evaluation by skin tests as a preliminary investigation seemed necessary. One hundred patients with seasonal symptoms of rhinitis and/or asthma were tested with extracts of two isolates of the ascospores from barley leaves. Four reacted to both extracts and eight gave a positive reaction to one of the ascospore extracts. It was noted that eleven of the twelve patients who reacted were already being treated for a mould spore asthma due to *Alternaria* or *Cladosporium*. One of these patients was admitted to hospital in severe status asthmaticus on July 29 at a time of high ascospore counts. Some patients with seasonal symptoms that seemed to be associated with barley had negative immediate skin tests, but one had positive precipitating antibodies present, indicating that these ascospores could also cause a disease of the "farmer's lung" type, caused by a Type III reaction of Gell and Coombs⁹.

It is remarkable that a fungus which is so abundant on barley leaves has not been studied before in Britain. In addition to its possible role as an aero-allergen, the question of whether *D. exitialis*, which is implicated by Müller¹⁰ as a cause of leaf scorch of cereals in Switzerland, damages cereal crops here also warrants investigation.

The late Dr Kate Maunsell suggested the Dorset study and Dr R. S. Bruce Pearson that near Cirencester. We thank Dr C. Booth and Dr E. Punithalingam of the Commonwealth Mycological Institute, Kew, for identifying the fungus and Mr B. G. Hart for the supervision of the Hirst spore trap at Blandford.

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¹ Frankland, A. W., and Davies, R. R., *Le Poumon et le Coeur*, 1, 11 (1965).

² Herxheimer, H., Hyde, H. A., and Williams, D. A., *Lancet*, i, 572 (1966).

³ Ganderton, M. A., *Acta allerg.*, 23, 173 (1968).

⁴ Hyde, H. A., and Adams, K. F., *Acta allerg.*, Suppl. VII, 159 (1960).

⁵ Gregory, P. H., *Rothamsted exp. Station, Rep. for 1972, Part 1*, 141 (1973).

⁶ Hirst, J. M., *Ann. Appl. Biol.*, 39, 257 (1952).

⁷ Last, F. T., *Trans. Br. mycol. Soc.*, 38, 457 (1955).

⁸ Corbaz, R., *Phytopath. Z.*, 66, 69 (1969).

⁹ Gell, P. G. H., and Coombs, R. R. A., *Clinical Aspects of Immunology*, second ed. (Blackwell, Oxford, 1968).

¹⁰ Müller, E., *Phytopath. Z.*, 19, 403 (1952).



Fig. 1 Deposit of airborne ascospores of *D. exitialis* ascospores on Hirst trap slide, exposed near Blandford, Dorset, on the night of August 1, 1972. Magnification: $\times 804$ approximately.

GENERAL

Turning the Left Cheek Examined using Modern Photography

McManus and Humphrey's investigation into turning the left cheek in formal portraits¹ from the sixteenth to twentieth centuries prompted me to check two 1972 school yearbooks for the same phenomenon. I found a similar tendency to expose the left side of the face more than the right. I found, however, no significant differences between sexes and seem to have discovered a difference between groups in left preference.

difference between the CWC faculty and staff and other groups, and also a difference between the CWC seniors and other groups, although no such tendency was noted in the Easley student body. The difference between the two Easley classes and the CWC student body was chiefly because of the CWC seniors.

If there really is a lessening of some sexual behaviour difference, or the development of group differences in facial exposure, Central Wesleyan College is one of the last places I would have expected to find such developments. Extreme hair length in males and other aspects of the "unisexual" look are discouraged. There is considerable social contact between faculty and staff and students, and both groups are largely drawn from the same small religious body

Table 1 Left/Right Alignment in Yearbook Photographs

	Left side of face exposed	Right	Left preference %	P*	Sex difference
CWC, senior males ²	18	12	60	NS	} <0.05
CWC, senior females	6	13	32	NS	
CWC, faculty and staff males	6	13	32	NS	} NS
CWC, faculty and staff females	4	9	31	NS	
CWC, all non-senior student males	49	35	58	NS	} NS
CWC, all non-senior student females	62	28	69	<0.001	
Easley, senior males ³	68.5	37.5	64	<0.01	} NS
Easley, senior females	68	30.5	69	<0.001	
Easley, sophomore males	57	26	69	<0.001	} NS
Easley, sophomore females	60	27	69	<0.001	

* P = Likelihood of these results showing preference, based on χ^2 test with 50% expected preference. Sex difference—based on 2 by 2 χ^2 comparisons. NS—not significant.

The photographs in the yearbook were taken by a single photographer for each school, who was unknown to the subjects. The subjects chose which of four poses would be published. It is possible that the same photographer took all the photographs used in this study. The use of a camera and subject selection of pose, and the same photographer for an entire group, seems to eliminate the hypothesis of bias in the artist's skill¹. Subject selection of pose would eliminate bias in positioning of the subject by the artist. Only head photographs were used in this study.

In Table 1 I show that preference was for the left side of the face to be exposed except for the three small groups from CWC. Because the senior females appeared to be different in preference, my wife independently checked senior males and females and found essentially the same preference tendencies as myself in both sexes (63% as against 60% in males, 27% as against 32% in females). As a further check against investigator bias or difficulty in classification, a naive observer independently classified the Easley seniors, and agreed almost perfectly with me (63.8% as against 63.6% in males, 68.6% as against 69.7% in females). The Easley seniors are an average of these two tabulations. No faculty head-only pictures were published in the Easley yearbook.

With the exception of the CWC seniors, no significant difference was found between sexes in any group, nor was there such a difference in all subjects combined, or all Easley students plus all CWC non-senior students combined. This difference from the findings of McManus and Humphrey might indicate a social factor in exposure preference¹, which has disappeared with time as some sex behaviour and appearance differences have decreased. It may also reflect fewer data in the present study.

In Table 2 I compare the several groups, using a 2 by 2 χ^2 test in each comparison. There seems to be a significant

Table 2 Pairwise Comparisons

	CWC, non-senior students	Easley, seniors	Easley, both classes	CWC, faculty staff
Easley, both classes	NS	—	—	<0.001
Easley sophomores	—	NS	—	—
CWC, all students	NS	—	<0.10	<0.01
CWC, seniors	<0.02	—	<0.01	NS

(the Wesleyan Church) in the Southeastern United States. Perhaps the group differences are statistical flukes, but it seems unlikely that the lack of sex difference in preference is, since it is reflected in both student bodies.

I thank Debbie Martin, Faye LaBar, Gilda Alexander, Donna Blair and Jimmy J. Kimble for technical assistance.

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¹ McManus, I. C., and Humphrey, N. K., *Nature*, **243**, 271 (1973).

² Easley, S. C., High School, *Green and White*, 45 (1972).

³ Central Wesleyan College, Central, S.C., *Centralian*, **38** (1972).

Reference Abbreviations

ALL abbreviations of references in *Nature* should now conform to the style of the *World List of Scientific Periodicals*, fourth ed. (Butterworth, 1963–65). Authors submitting manuscripts are asked to ensure that the references are written appropriately.

BOOK REVIEWS

Sex in the Making of Man

Sexual Selection and the Descent of Man, 1871-1971. Edited by Bernard Campbell. Pp. x+378. (Heinemann: London, June 1973.) £6.50.

DARWIN'S *The Descent of Man and Selection in Relation to Sex* contained, as its name suggests, two largely independent works. The two parts are held together by Darwin's conviction that many of the differences between the races of man cannot be explained as adaptations to different environments (and hence by natural selection), and therefore must be explained by sexual selection, and in particular by different concepts of human beauty. Few people today would take this argument very seriously. One might therefore fear that a collection of essays celebrating the centenary of Darwin's book would lack unity to an even greater extent than most such collections. In fact, this fear is not realized; these essays form a sustained and coherent attack on a related group of problems. The credit for this must go in part to the editor, Bernard Campbell. He has chosen his authors skilfully, and has given each of them space to develop his ideas. It is also in part the result of the happy accident that history has brought the two parts of Darwin's book closer together. We can now see that if we are to understand human evolution, we must also understand the evolution of human breeding systems, and the relevance of these systems for ecology and sociology on the one hand and for individual psychology on the other.

In the first two essays, Eiseley puts Darwin's work in historical perspective and Simpson summarizes what is known of man's physical evolution. This is followed by an essay by Campbell himself, relating man's physical evolution to his ecological status. In brief, he suggests that *Australopithecus* was confined to a tropical environment, that with *H. erectus* man's ecological range was extended to temperate regions, and with *H. sapiens* to the Arctic. There follows a clear summary by Dobzhansky of the genetics of human racial differences, and an essay by Mayr introducing the problem of sexual selection. The latter seems to me a sad and uncharacteristic muddle. The phrase "sexual selection" has been applied to many different selection processes, and Mayr sets out to distinguish these from each other and from natural selection. Unfortunately he never gets clear about

the most fundamental distinction—that between intra- and interspecies selection—and ends up by apparently equating natural selection to the latter. "I feel that here is an area that has not been thought out completely", he writes; he can say that again.

Ehrman reviews the evidence on the genetic basis of sexual selection, and in particular her own fascinating work on the "rare male" phenomenon, whereby a female fruitfly, offered a choice of kinds of male, will prefer to mate with whichever kind is least common. There follow three brilliant essays, by Trivers, Selander and Crook, on the significance of sexual selection; together these form the essential core of the present volume. Trivers's fundamental theme is that parental investment of the sexes is the key variable controlling the operation of sexual selection. The argument seems to me correct and illuminating. The essay is densely packed, both with theory and data, and is not always easy to follow; I hope he will expand it into a book. I think he makes some mistakes; for example, can it be true that "American women tend to marry up the socioeconomic scale", unless upper class American women stay unmarried? (What is true—at least in Scotland—is that tall women tend to marry up the scale.)

Selander starts with a review of Darwin's own contribution, including a sympathetic discussion of his mistakes. He then reviews sexual dimorphism and selection in birds. His central argument is that ecological requirements determine breeding systems, and that these in turn influence sex ratios and sexual dimorphism. This essay too is hard work because so full of illustrative data, but again the effort is worth it. Crook discusses sexual selection in primates, and, like Selander, interprets breeding systems as ecological adaptations. He is less convincing, because as yet less is known of the ecology and ethology of primates than of birds, but his essay is packed with fascinating problems. For example, he describes how, in the Old-World monkeys, males often mimic female genital characteristics as a method of warding off aggressive attacks from dominant males. But why should it be that in the New-World monkeys, the male genitalia are sometimes used as signals in aggressive inter-male displays and are mimicked by displaying females? Crook discusses

the role of sexual selection in human evolution. Here the fundamental difficulty is that we do not know the breeding system of our recent ancestors. Crook discusses Desmond Morris's thesis that much of human evolution can be understood as a series of adaptations making sexual intercourse more rewarding and hence cementing the pair bond. Crook finds the idea plausible, but remarks that "the fact that Morris's story hangs together particularly well may tell us more about his skill as a writer than about the validity of his views". This leads him to discuss how evolutionary hypotheses are to be validated, and to suggest that they must be tested at levels additional to that which originally suggested them.

This last methodological proposal might be pondered by Fox, who contributes an essay on sexual selection and human kinship systems. The essay contains many interesting suggestions, but suffers from the fact that the author is obsessed by a single idea. He believes that the human neocortex, the human conscience and sense of guilt, and human kinship systems and incest taboos, owe their existence to sexual selection operating between males in a society in which only dominant males get mates. Like most obsessions, this is part of the truth; if it were the whole truth, it would explain why only man, and not dogs or baboons, invented Christianity. I am also curious to know how Miss Greer will react to the suggestions that women owe their intelligence to selection between males for dominance.

The book ends with a useful summary by Caspari of what has gone before. In an apparent attempt to redress the balance between the sexes, he ascribes my work on group and kin selection to my wife. To conclude, this volume is the most informative and stimulating discussion of sexual selection at present available.

J. MAYNARD SMITH

Free Radicals

Free Radicals. Volume 1. Edited by Jay K. Kochi. (Reactive Intermediates in Organic Chemistry.) Pp. xvii+712. (Wiley-Interscience: New York and London, April 1973.) £18.75.

SCIENTIFIC progress depends almost as much on the formulation and critical examination of theories as on the discovery of new facts. Theories cannot be computerized and, since the present

output of scientific papers in free radical chemistry is already far too great for thorough comprehension by personal reading, specialist review volumes, though expensive, have become essential secondary sources of information for research workers. Monograph series, such as the Wiley-Interscience publications, of which this is but one volume, are thus necessary additions to the shelves of reference libraries.

Even the poorest specialist essay provides its readers with references that save days of working time in the preparation of a card index: a good essay in addition stimulates thought and the critical appreciation of the merits, failings and basic objectives of modern research papers. This collection of eleven essays on free radical reactions is a mixed bag in regard to both quality and intrinsic value. Each author has been allowed to present his own view of the scientific importance of his selected topic. Some (for example, J. W. Wilt, who contributes a mammoth chapter on free radical rearrangements) have chosen to write up a comprehensive card index, others present narrowly American viewpoints on small and highly specialized researches (for example, R. G. Bergman, who describes biradicals by detailed reference to trimethylene, and J. G. Garst of the University of Georgia, who discusses radical-anions mainly by reference to that of naphthalene), but the more experienced writers tabulate data and discuss critically their overall value. These chapters are of general interest because they show that few novel kinetic theories are now likely to emerge and that far too many of our published data are experimentally imprecise and often theoretically superficial.

This last comment applies particularly to the chapters by J. D. Kerr on rate constants in gas phase reactions, by K. U. Ingold on absolute rate constants in liquid phase reactions, by G. A. Russell on relative rate constants of radical reactions and to two short chapters by T. Koenig on some typical homolytic decompositions and on "cage effects" on decomposition rate in solution. J. K. Kochi presents kinetic researches much more descriptively. He deals with redox reactions of free radicals and metal complexes, covering the wide field of his own recent interests but including in some detail well known theories of metal ion catalyses of autoxidation and peroxide decomposition. A. G. Davies and B. P. Roberts, in a concise but stimulating chapter on bimolecular homolytic substitution at metal centres, extend widely the mechanistic outcome of their own work on the free radical chemistry of boron, phosphorus and sulphur. The outstanding new development in free radical chemistry is also described in a short chapter

on chemically induced dynamic nuclear polarization (CIDNP) by H. R. Ward. This new physical technique for the diagnostic detection of free radical reactions has great potentialities particularly for the study of reaction mechanisms, but Ward has been quite cautious in his assessment.

The book as a whole shows that many aspects of free radical chemistry have now been developed so extensively that they are declining in academic but not industrial interest, though they still comprise the efforts of the majority of research workers in this field. However, some quite new themes and experimental techniques are emerging and these have quite properly been brought to our attention.

W. A. WATERS

Thermodynamics

An Introduction to Equilibrium Thermodynamics. By Bernard Morrill. Pp. xi+353. (Pergamon: Oxford and New York, February 1973.) £7.50.

I AM a chemist by training and was somewhat disconcerted, having accepted an invitation to review on title alone, to find that this volume is slanted towards American engineering students. Nevertheless, it is interesting to discover how others learn. The book's introduction suggests that the text is intended to "open up new areas of electrical engineering thought". I expected, therefore, a thermodynamic discussion on thermocouples and thermoelectric power, Peltier effects, Johnson noise, fuel cells, and so forth. None of these is mentioned and the most specialist treatment is reserved for steam engines, steam turbines and nozzles.

The main text covers a wide range of traditional thermodynamics, Carnot cycles, Gibbs functions, ideal gas mixtures and the like, which are at least as familiar to chemists as to engineers. The treatment takes the principles of the subject quite rapidly so that the third law and grand partition functions are completed by the end of chapter 2, to be followed by some more detailed work and the applications. The text is, in general, competent, clear and fairly mathematical. I improved my knowledge, especially from the section on Legendre transformations and from the treatment of throttles. But there is nothing exceptional for those content with their present texts.

There are problems, both worked and for the student. It is in the adoption of Imperial units that most is done to make the work especially suitable for engineers. I hope I shall never again meet the universal gas constant in the form $1545.3169 \text{ ft lbf/}^\circ\text{R lbm mole}$.

D. H. WHIFFEN

Phonons

The Physics of Phonons. By J. A. Reissland. Pp. xi+319. (John Wiley: New York and London, April 1973.) £7.

THIS book is based upon an excellent idea: to present in one volume most of the aspects of phonon physics; and so it is even more restricted than Ziman's *Electrons and Phonons*. As a reference book for undergraduates, graduate students, as well as research workers, it presents a very convenient compendium. I would, however, hesitate to recommend it to my own students as a text, especially if it were to be the first text. The book is very light on discussion of the important—and usually very difficult—concepts. Why is a phonon called a quasi-particle? Why the name Umklapp-process? What is created by a creation operator? Why are some particles bosons and some fermions? None of these questions is answered—or asked. This is a great pity because a useful book could have become a classic. It would also have been worthwhile to discuss and underline the similarity and difference with other quasi-particles (or "ons", as the author calls them in chapter 8).

The coverage of the book is comprehensive and can hardly be faulted. Through a discussion of normal vibrations phonons are introduced in chapter 1. Chapter 2 discusses dispersion curves and in chapter 3 creation and annihilation operators are introduced. In chapter 4 Reissland discusses statistics without, however, really making clear the distinction between various kinds of statistics—after having stated categorically that all particles are bosons or fermions, he mentions that the atomic oscillators follow Boltzmann statistics. Anharmonicity and phonon-phonon interactions are introduced in chapter 5, and the Green function formulation follows in chapter 6. Chapter 7 is devoted to a lattice dynamical treatment of various solid state properties, such as thermal expansion, ultrasonic attenuation, and ferroelectricity. In chapter 8 the interaction of phonons with other "ons" is discussed, but I would have expected more than a seven-line paragraph devoted to superconductivity and at least a brief description of the basic ideas (such as the importance of Cooper pairs) would have been welcome.

The book is well produced—although a few names are mis-spelled—and as present-day prices go not too expensive. The author does not mention whom he had in mind when writing the book. I should have thought that it was primarily intended to help starting graduate students in solid-state research, or even the final-year undergraduate, and the copious references will be helpful in this respect. A selection of exercises would have been welcome.

D. TER HAAR

Vital Statistics

Biometrical Interpretation. By N. Gilbert. Pp. 125. (Clarendon Press: Oxford; Oxford University Press: London, May 1973.) £3 boards; £1.30 paper.

Introduction to Biostatistics. By R. R. Sokal and F. J. Rohlf. Pp. xiii+368. (W. H. Freeman: San Francisco, May 1973.) £4.10.

As stated in the preface to *Biometrical Interpretation*, "this book is addressed to biologists who use biometrical methods as a tool". Instead of justifying mathematically, or leaving unjustified, the statistical methods presented, it describes plainly, thoroughly and concisely how a set of quantitative biological data can be broken down and analysed to show its salient structural features. In a properly designed study these features will correspond as answers to the biologist's questions and thus provide a natural basis for interpretation. This book is therefore refreshingly different from the many "recipe books" already available for the relatively non-numerate user of routine statistical methods. Just because it is written to give the user an intelligent understanding of the way in which an appropriate statistical analysis has built in to itself the structural features of his particular problem, it is less easy to read (but far more rewarding) than a cook-book of bland instructions and formulae.

Although from the preface "the book assumes some familiarity with elementary statistical methods", the author has in fact explained most of the important ideas himself where necessary. In the first six chapters a unified treatment is adopted, centred on least squares estimation of linear relationships and additive effects in the presence of Normally distributed residuals. Discussion of means and variances is naturally followed by an account of correlations, multiple regressions and prediction. A minor criticism is that principal component analysis is described (page 40) as attempting to summarize the correlation, rather than (as I think is conventional) the covariance, structure of a set of variables; readers may therefore find the discussion of principal components and factor analysis confusing. The importance of assumptions of additivity and linearity is clearly stated: common transformations to linearity and additivity are described, as is the use of polynomial and other mathematical functions—some useful notes on curve fitting are also given in chapter 13. Significance testing is explained, with examples of normal theory tests on means, variances and contingency tables.

Chapters 7 to 11 cover varied topics. The Fisherian and Bayesian views of

basic statistical theory are contrasted in chapter 7 and we are warned to temper with common sense our adherence to any particular approach. Chapter 8 stresses randomization and the appropriate design of experiments. Chapters 9, 10 and 11 discuss briefly uses of statistics in problems of particular interest to biologists: indices of diversity, quantitative genetics and population dynamics. No account is given of probit analysis, however. The book ends with a plea for correct analysis (or else no analysis at all), a valuable summary of how to tackle a specific problem by quantitative experimentation, and lucid answers to most of the 40-odd examples set. Whoever masters this slim volume should have a clear and sound understanding of most basic statistical methods and the circumstances (not only biological) in which each is appropriate. I strongly recommend this book.

I find little, on the other hand, to distinguish *Introduction to Biostatistics* from many other introductory texts.

It is an elementary introduction to statistics, illustrated by examples drawn from biological applications, and requiring only an elementary knowledge of mathematics. The layout of the book is conventional: it begins by explaining the nature of quantitative data and how their distributions may be summarized by descriptive statistics. Next, simple probability theory is followed by discussion of the binomial, Poisson and normal distributions. Estimation, confidence intervals and tests of hypothesis are discussed, mainly in the context of normally distributed data. One and two way analyses of variance are discussed, with the inclusion of a chapter dealing with the assumptions underlying such analyses, possible transformations of the data and the use of two-sample and paired-comparisons nonparametric tests. Other topics treated are simple linear regression ($y=a+bx$), simple correlations, and χ^2 tests of goodness of fit and independence in contingency tables.

A possible disadvantage of this book is that answers are given to only a few of the examples set; however, many worked examples are presented with full details throughout the text. On the credit side, I welcome the distinction made between hypotheses formulated before and after inspection of the data (page 179). In the discussion of tabular χ^2 tests, the use of the log (likelihood ratio) statistic (page 293) instead of the traditional χ^2 statistic (page 290) is an interesting innovation. For an elementary text the book also contains a wide range of statistical tables. Although about three times as long as *Biometrical Interpretation*, it compares if anything unfavourably with Gilbert's book in respect of the extent and depth of

ground covered. For a discursive and informal presentation, the longer book might be preferred. Among users of statistics, however, not only the researcher but also the competent undergraduate would in my view find the shorter and cheaper book far better value.

K. L. Q. READ

Flowers of the Field

An Ecological Atlas of Grassland Plants. By J. Philip Grime and Philip S. Lloyd. Pp. 192. (Edward Arnold: London, 1973.) £6.

THIS book collates data from an intensive survey of grassland sites in the Sheffield region of Northern England. The authors adopt an autecological approach to vegetation, so that the book tends to be an analysis rather than a synthesis of grassland types. For each of the ninety-four species considered an attempt is made to correlate distribution and frequency with a number of environmental variables.

The survey covered sixty-seven sampling sites in which a total of 630 m² quadrats were selected at random. The sites included a variety of geological substrates, including limestones, millstone grit and bunter sandstone. Within each quadrat a "frequency" value was obtained for each species, being the percentage of 10 cm × 10 cm subunits in which the species was found rooted. These data form the basis for the analysis of the requirements of the individual species which comprises the bulk of the book.

Summaries of the data available for each species are set out most clearly in the form of tables (covering pH variations on the various geological substrates), histograms (showing species constancy against surface pH and slope) and whole page ordination diagrams in which species frequency is shown on aspect/slope axes. These display a great deal of information in a visual form which is very easy to interpret. The book is thus not an atlas in the conventional sense of demonstrating spatial distributions of species, but it does construct spatial models which illustrate ecological preferences and limits.

Perhaps the greatest weakness of this book is its title, which possesses encyclopaedic, authoritative overtones not justified by its contents. Although the grassland survey is intensive, it is limited in its geographical extent. As a result one must be extremely cautious in applying its findings to other parts of the country. For example, *Viola hirta* is recorded as a species of southerly aspect by this survey. This may be true in the Sheffield area, where this species of southerly geographical distribution could be under climatic stress. In South-

east England it is commoner on northerly aspects. Again, the conclusion that *Hypochoeris radicata* is confined to base rich, calcareous soils does not appear to apply to the distribution of this species on the North Downs of Surrey and Kent, where it often indicates the presence of acidic deposits of clay with flints over the chalk.

Grime and Lloyd neglect the consideration of general height of vegetation as a microclimatic determinant for species of small structure. Often this is as important as aspect in controlling the distribution of such species. Could the fact that *Medicago lupulina* (a prostrate species) is restricted to southerly aspects in the survey simply reflect the preponderance of short turf vegetation on south facing slopes?

This volume contains an abundance of interesting information on the behaviour of grassland species in the Sheffield area. Its application in other areas should be undertaken with considerable care. PETER D. MOORE

Introducing the Hamiltonian

Advanced Molecular Quantum Mechanics: An Introduction to Relativistic Quantum Mechanics and the Quantum Theory of Radiation. By R. E. Moss. (Studies in Chemical Physics.) Pp. xvi + 300. (Chapman and Hall: London; May 1973.) £5.90.

THE scope of this book is conveyed less accurately by the title than by the subtitle—*An Introduction to Relativistic Quantum Mechanics and the Quantum Theory of Radiation*. The reader interested in advanced non-relativistic theory should therefore be warned that he will find no discussion of the general area of many-electron quantum mechanics or of theories and techniques for calculating electronic wavefunctions. That being said, Dr Moss is to be congratulated on producing a timely and outstanding monograph in its field.

The book is concerned almost exclusively with the establishment of the many-particle Hamiltonian, including all the "small terms" arising from electron and nuclear spins and internal and applied fields—terms whose effects are nowadays measured with considerable precision by electron and nuclear resonance techniques. The relativistic origin of such terms is imperfectly understood by many of the chemical physicists who take their existence for granted; so also is the quantum field theory needed for a proper treatment of the interaction between a molecule and the radiation field. An acceptable treatment of this area, in the form of a self-contained and up-to-date monograph, was lacking: this text fills the gap admirably.

The reader is assumed to have a know-

ledge of elementary non-relativistic quantum mechanics, of which he is reminded in the first ten pages. The next five chapters contain a fairly detailed account of all the matrix algebra, classical mechanics, relativity and electromagnetic theory needed in the second half of the book. Brief but clear explanations often replace a rigorous discussion; but those who find the account a little terse (relativity theory in fourteen pages) will find themselves easily persuaded, and better equipped, to consult the references.

The next part of the book contains an introduction to relativistic wave equations; a good account of the Dirac equation and its reduction to Pauli form; a simplified discussion of the Breit equation and its Pauli form, for a many-electron system; and finally the introduction of nuclei to give the full "molecular Hamiltonian". Unfortunately, the link between this Hamiltonian and the effective or phenomenological Hamiltonian used by spectroscopists receives very little attention. The only application is a chapter on the relativistic theory of the hydrogen atom.

The two remaining chapters contain a simple and well-written introduction to the quantization of the radiation field and its inclusion in the Hamiltonian, leading to the theory of absorption and emission.

Important features are the use of SI units throughout and the high standards of accuracy achieved in reproducing elaborate equations; much checking revealed remarkably few errors or misprints. The quality of production is high and the book deserves to become a standard reference in its field.

R. McWEENEY

The Natural Balance

Ecology. By Robert E. Ricklefs. Pp. x+861. (Thomas Nelson: London, September 1973.) £3.95.

THOSE concerned with the conservation of our forests may well be worried by the present plethora of books on the environment and ecology. It is difficult to conceive of a population crash, but one might perhaps hope that competition may slow down the reproductive rate.

Ecology is becoming a word so broad in meaning that it is difficult to guess what a book on this subject might cover. The present work is particularly broad in approach, covering as it does subjects as diverse as perception, time sense, basic properties of the physical environment (these as separate chapters in a part entitled "Ecology of Organisms: Physical Environment"). One aspect which it does not emphasise greatly is the effect of man, pollution, and so forth. The work starts by showing how easily and

unknowingly man can upset the environment, but the book is for the biologist, not the environmentalist, and the matter is not laboured.

Apart from the perhaps over-broad approach, the most unusual aspect of the book is the large amount of space devoted to evolutionary aspects of animals and plants. Too many books on ecology tend to ignore the evolutionary aspects of the subject, but this work emphasises them strongly. The author divides the book into eight parts encompassing forty-six chapters, and of these, three parts and sixteen chapters are about evolution in its broadest sense (the parts are called "Natural Selection and Adaptation", "Ecological Genetics and Evolution", "Genetics of Populations"). While I warmly welcome the approach that evolution and ecology are closely interwoven, some of the subject matter in these chapters is again perhaps rather far from the title of the book (for example, a chapter on the rate of evolution).

The other parts cover ecology of organisms, biological environment, ecology of populations and ecology of communities. The first of these three covers predator and prey relationships and competition, as one might expect, but also covers social environments and sexual relationships (including courtship and territory), together with a chapter on the social insects. The part on populations covers population regulation, predator-prey interactions, and evolutionary responses. The part on communities deals with community ecology, energy flow in ecosystems, relative abundance of species, and species diversity.

The book is clearly written, based around the numerous examples. There is an abundance of clear text figures and photographs, including a particularly pleasing 20-page section of photographs of the vegetative communities of the world. There are nearly 40 pages of references which should enable almost anyone to make a start on the literature on any of the subjects covered. The index is well done, though I should have liked it to include the names of the authors cited. There is relatively little mathematics in most of the book, though it appears in the chapters on population regulation, competition and predator-prey interactions; it should seldom prevent the general reader from understanding the subject.

The general reader will, however, find the work rather long; nevertheless, by selecting those chapters of particular interest he should be able to get a good understanding of the subject. The student wishing to get a broad understanding of the life of whole organisms, especially animals, will find a great deal of interest in this work.

C. M. PERRINS

CORRESPONDENCE

Conferences in Russia?

SIR,—Two events occurred this summer which, taken together, should be of particular concern to geneticists, as well as many other scientists. On July 16 the Supreme Soviet deprived Dr Zhores Medvedev of his Soviet citizenship, thus preventing his return from Britain to the USSR. On August 23 it was announced at the 13th International Congress of Genetics at Berkeley, California, that the International Genetics Federation had recommended that the next International Congress should be held in Moscow in five years time.

Dr Medvedev's reputation is based both on his scientific research and his courageous attempts to expose some of the defects in the organisation of science in the Soviet Union. His sole purpose throughout has been to help eradicate these defects. One of his books¹ provides a detailed history of the Lysenko period, when a deliberate and systematic attempt was made to kill the science of genetics. This book has not been published in the Soviet Union, and many supporters of Lysenko still hold influential positions in biology. Another book² deals with the difficulties he and other Soviet scientists have had in trying to communicate with or visit their colleagues in other countries, and with the problems of censorship. This book has not been published in the Soviet Union either, and there is every indication that the situation is now worse than it was when Dr Medvedev described it in 1970. By publishing these books in the West, he has brought to light facts which are unpalatable to the Soviet government, and it is certainly for this reason that he was deprived of his citizenship.

Scientists should support Dr Medvedev in his struggle to break down the barriers to communication, but they should not assume that attending meetings or congresses within the Soviet Union necessarily provides such support. It is essential to realise the hypocrisy of the official Soviet attitude to free exchange of scientific ideas and information. In effect, the Soviet Union has for many years imposed a boycott on numerous scientific meetings or conferences outside Eastern Europe. Anyone who has organised conferences in the West and has invited a Soviet scientist to participate knows that the invitation will often be either refused or ignored. If it is accepted, in most cases

the scientist does not in fact come to the conference. In many instances this has caused considerable confusion and has prevented some other well qualified scientists from participating.

Under these circumstances, why should one take seriously the attempt by the Soviet organisers to bring about exchange of information between scientists at an international congress in Moscow? Furthermore, why should other Genetical Societies collaborate with the Society organising the Moscow congress (N. I. Vavilov's All-Union Scientific Society of Genetics and Selection) when that body has not protested against the exile of one of its best known members, who has done so much to reinstate genetics as a real science in the Soviet Union?

I believe that geneticists should attend the congress in Moscow only if the following conditions are fulfilled. (1) The Academy of Science of the USSR must guarantee that there will be no restriction to participation on political or any other non-scientific grounds. (Unfortunately the verbal statement to this effect by the Soviet delegate at the International Federation meeting at Berkeley is not an adequate assurance.) (2) There must be some evidence within the next few years that Soviet scientists who are invited to conferences in the West will be allowed to attend. (3) Dr Medvedev's citizenship must be restored.

Following the decision at Berkeley, I think it is very important to find out whether geneticists in countries outside the Soviet Union share or disagree with my point of view, and I would therefore welcome letters which express a clear opinion on this matter.

Finally, I must make it absolutely clear that although Dr Medvedev and I are collaborating in ageing research in the Genetics Division of this Institute, this letter was written entirely on my own initiative, and he is in no way responsible for any part of it.

Yours faithfully,

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¹ Medvedev, Zh. A., *The Rise and Fall of T. D. Lysenko*, trans. by Prof. I. M. Lerner (Columbia University Press, New York, 1969).

² Medvedev, Zh. A., *The Medvedev Papers*, trans. by Vera Rich (Macmillan, London, 1971).

OUR correspondent, W. H. McC., writes:

SIR,—In the London telephone directory there are some tens of thousands of entries having the first initial H and a similar number having a second initial W, and there are over a hundred entries with the name Davenport. There is, however, as it happens, precisely one H. W. Davenport. Here, for the satisfaction of the H. W. Davenport who wrote (from Michigan unfortunately) the letter you published in *Nature* (245, 111; 1973) is a case where "three doubts make a truth".

In the sentence he quotes from my notice, as I had stated, "item" has a technical meaning assigned by Sturrock in the work I was reviewing. So the quoted sentence has a more sophisticated significance than appears when it is taken out of context. Nevertheless the result is basically the same as the probability of identifying someone by name and initials. I did, in fact, comment that the result was expected but that Sturrock's quantitative illustration was highly interesting.

Paperback Polymorphism

SIR,—I find to my astonishment that I have three copies of the Dover paperback edition of R. A. Fisher's *The Genetical Theory of Natural Selection*. The books are identical in every respect except that the cover of one is green another red, and the third grey—colours which contrast conspicuously and which perhaps are responsible for my having inadvertently bought three copies. Fisher himself was deeply interested in the theory of genetic polymorphism, and no doubt would have been delighted to hear that the polymorphism in the paperback edition of his classic work had possibly affected its sales. It would be interesting to know if by producing the same book with different coloured covers affects the search image of a potential purchaser in such a way as to increase sales.

Yours faithfully,

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Anomalous Water

SIR,—The note on the 'Nature of "Anomalous Water"' by B. V. Derjaguin and N. V. Churaev¹ is not con-

vincing. Many observations reported by Derjaguin's school cannot be accounted for by the presence of minute amounts of impurities. Thus, density, viscosity, and heat conductivity of anomalous water as given in *Issledovaniya v Oblasti Poverkhnostnykh Sil* (Studies on Surface Forces)² deviate from the corresponding properties of ordinary water so much that no traces of impurities

such as silica can explain the deviation. The protracted error immortalised in scores of publications can be due only to psychological (self-deception), not to chemical causes. The 'anomalous' water belongs to the category exemplified by the N rays of Blondlot³.

Yours faithfully,

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¹ Derjaguin, B. V., and Churaev, N. V., *Nature*, **244**, 430 (1973).

² *Issledovaniya v Oblasti Poverkhnostnykh Sil* (Studies on Surface Forces), 3, 31, 44 (Moscow, 1967).

³ Blondlot, R., *N Rays* (Longmans, Green and Co., London, 1905).

Obituary

Professor N. K. Adam

NEIL KENSINGTON ADAM, FRS, who died in Southampton on July 19, was born 81 years ago, son of Dr James and Mrs Adela Marion Adam of Cambridge, and eldest of a family of three—his brother Arthur died in the 1914–18 war; his sister Barbara (Lady Wootton) survives him. After attending Winchester he went to Trinity, Cambridge, of which he later became a Fellow (1915–23). From Cambridge he undertook wartime chemical service with the Royal Naval Airships Service. But it was in 1921 that he commenced his distinguished researches into insoluble monomolecular surface films, being awarded in that year the Royal Society's Sorby Research Fellowship at Sheffield.

Inspired by Langmuir's earlier work, he (with G. Jessop) designed and built a series of surface pressure balances capable of measuring to about 0.01 dyne cm⁻¹ (10⁻⁵ N m⁻¹), using the simplest of materials: a brass trough, waxed glass strips, phosphor bronze wire, a glass pipette, an optical lever and a ruler. Another eminent surface chemist has aptly described this as "measuring molecules with a metre stick". During the 1920s and early 1930s he was responsible for a long series of Royal Society papers documenting the surface behaviour of a very wide range of amphipathic insoluble monolayers. He was thus able to explore a new two-dimensional world with its own equations of state, analogous in many ways to three-dimensional solids, liquids and gases, but with some intriguing differences. The extension of this work to biological materials, e.g. sterols, enabled him in 1929, with O. Rosenheim, to provide powerful evidence in favour of the cyclopentenanthrene structure of cholesterol, a view further supported by Bernal's X-ray analyses at about the same time. N. K.'s tenure at Sheffield culminated in the appearance of the first edition of *The Physics and Chemistry of Surfaces* which, besides chronicling the whole subject of insoluble monolayers, dealt with most other aspects of interfacial chemistry and physics.

After Sheffield, he moved to London to work at University College on the then new subject of colloidal electrolytes which later became the basis of modern detergents. During this period he was chiefly concerned with the surface properties of solutions of pure anionic and cationic surface active substances and their relationship to micelle formation. Nevertheless, monolayer studies, particularly of biological materials, such as sterols, oestrogens, celluloses and proteins, were continued by a succession of students whose names became familiar in the surface chemistry and biology journals. In 1935 he was elected FRS. The second edition of *The Physics and Chemistry of Surfaces*, which was almost a complete re-write, appeared at the end of the University College period, and he moved to Southampton in the autumn of 1937.

The situation at Southampton, not yet a university, was very different from that at University College, where Donnan had been Head of the Chemistry Department, with Ingold occupying the chair of Organic Chemistry: Freundlich was also there. N. K. found himself Head of a department with two lecturers, one assistant lecturer, two demonstrators and a research assistant occupying premises which included two huts from the 1914–18 war. Within two years war had come again, and although the college escaped with comparatively little damage, the problems of maintaining the department in a city that received so much of the enemy's attention are difficult to overestimate. Nevertheless he took a leading part in the ARP activities, such as fire watching and gas detection, and yet found time to get the third edition of *Physics and Chemistry of Surfaces* published in 1941. After the war, with the growth in demand for university places, N. K. was preoccupied with planning new accommodation for the Chemistry Department and became more and more involved in college administration leading up to the granting of university status in 1952. He was Dean of the Faculty of Science for many years, and of this period he wrote that administrative chores were "time consuming, though not always uninterest-

ing". Just before his retirement in 1957, he published a second textbook, *Physical Chemistry*, written with the same clarity and accuracy that were characteristic of the earlier *Physics and Chemistry of Surfaces*.

Having detailed, very inadequately, his academic life, one has only told part of the story of this very remarkable man. His dedication to the task in hand, whether it be planning for a new laboratory, preparing a lecture, or adjusting the tappets of his car (which was new in 1934 and lasted almost to the end of his life); his addiction to "do-it-yourself", which extended into the laboratory; his abhorrence of pretension and insincerity; his fiery temper; his sense of fun; his kindness and sensitivity; his generosity; his love of music; his passion for ducks—all these endeared him to those who enjoyed his friendship and now mourn his passing. His wife, Winifred, whom he married in 1916, and with whom he shared so many of his interests, and their son Arthur, survive him—their daughter Jean (Mrs Adams) died several years ago.

The funeral service, conducted according to the practices of the Church of Christ, Scientist, had a simplicity that was entirely appropriate to everything that N. K. had stood for.

Addendum

IN the article "Effect of Commensal Hydroids on Hermit Crab Competition in the Littoral Zone of Texas" (*Nature*, **241**, 139; 1973) by H. O. Wright, the author has informed us that the name of the junior author and the acknowledgments were inadvertently omitted from the revised manuscript. G. A. Matthews should have been listed as junior author and the acknowledgments should have read as follows: "Part of the background work was performed by S. Brunenmeister, C. Shaw and other University of Houston students. We thank C. Hand for suggesting the problem, based on an old personal communication by M. Burkenroad."